IDF Symposium on Cheese

Prague, Czech Republic
March 21–25, 2004

Ripening, Characterization & Technology

Book of Abstracts

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ABSTRACTS
OF ORAL PRESENTATIONS

SPRAY DRYING IN THE CHEESE INDUSTRY

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The paper is dealing with the application of spray drying in, and in the relation to the cheese industry. In the introduction the technology of producing the cheese powder is briefly described and also the production of the skim milk powder suitable for later production of cheese. Also the technology of whey processing to various dry products is briefly discussed. The main subject of the paper is the description of a new process, called TIXOTHERM™, developed recently by Niro, for the processing of permeate, produced as a by-product from the ultrafiltration of whey, into a non-hygroscopic powder. After evaporation to 60% TS the permeate concentrate is subjected to a three step process consisting of concentration to 86% in the Rosinaire™ paddle dryer, holding, stabilization and curing in a screw conveyor with two augers, and finally drying and cooling in a combined back-mix/plug-flow fluid bed. In comparison with the traditional processes the TIXOTHERM™ provides great savings of energy (about 30%) and building costs (up to 75%).

CHEESE AUTHENTICITY AND TRACEABILITY: AN ANALYTICAL CHALLENGE

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Emmental or Swiss cheese is one of the most known cheese variety worldwide. It is produced in many industrialised countries at a very different price. To fight or prevent fraudulent labelling of this cheese type, pertinent tools for geographic origin assignment are needed. For this purpose, cheese samples from the main European manufacturers (n = 15 for Austria, n = 12 for Finland, n = 63 for France, n = 23 for Germany and n = 70 for Switzerland) were collected, corresponding to 110 winter and 73 summer samples.

From a preliminary study, a series of promising analytical methods were applied to these 183 samples to measure the following parameters: total nitrogen, water soluble nitrogen, 12 % TCA soluble nitrogen, pH-value, volatile short chain acids, sodium, chloride, organic acids, microorganisms, trace elements (Cu, Zn, Mg) and stable isotope$_2$ ratios (δ^2H, δ^{13}C, δ^{15}N, δ^{34}S). In a first step, analysis of variance (ANOVA) was applied with the the two factors geographic origin and the season of production. Significant differences between the regions of origin were found for all the parameters mentioned above (p = 0.001). The criteria acetate, propionate, capronate, water soluble nitrogen, 12 % TCA soluble nitrogen, pyruvate, obligate heterofermentative Lb. (OHL), Zn and δ^{13}C showed significant differences according to the season in certain regions.

In a second step, the data was treated by multivariate statistical analysis. Various pattern recognition algorithms (discriminant analysis, general linear model (GLM), neural network) were compared to classify the Emmental samples according to their geographic origin whereas the main accent was put on the differentiation of the Swiss Emmental from those manufactured in other countries. The former could be 100% correctly identified combining the following parameters by pairwise comparison in a GLM model: volatile short chain acids, total nitrogen, pH-value, D-lactate, succinate, L-leucin-aminopeptidase, pyruvate, OHL, δ^2H, δ^{13}C, δ^{15}N and δ^{34}S.

Keywords: Cheese Authenticity, Emmental, multivariate statistic

SPECIES IDENTIFICATION IN CHEESE VARIETIES USING ELECTROPHORETIC, CHROMATOGRAPHIC AND PCR TECHNIQUES

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In Europe, cheese varieties made from ewes' and goats' milk are of considerable economic importance as a result of widespread acceptance of traditional cheeses. However, the substitution of cows' milk for ewes', goats' and buffaloes' milk is a fraudulent practice in the dairy industry.

In Austria, mixed cheese varieties are produced either from cows' and ewes' milk or from cows' and goats' milk; in both cases the maximum percentage of cows' milk allowed is 49%. In addition, some cheeses are manufactured from cows', ewes' and goats' milk (minimum 10% of each species). Therefore, food analysts are challenged not only by milk species identification (e.g., the qualitative detection of cows' milk), but also by the need for quantitative determination of cows', ewes' and goats' milk in mixtures.

Using standard mixtures of milk as well as model cheeses (Camembert, Tilsit, Kashkaval) of different ages, quantitative determination of the percentage of cows' milk in mixed-milk cheeses was performed by isoelectric focusing (IEF) and subsequent densitometric evaluation of γ-caseins (Cn). Because ewes' and goats' milk cannot be distinguished by this method, bovine, ovine and caprine milks were differentiated using IEF and cation-exchange high performance liquid chromatography (HPLC) of para-κ-Cn. Species identification by polymerase chain reaction (PCR) using species-specific primers was applied to compare the detection limits of protein- and DNA-based methods, respectively.

The analytical procedures used were appropriate for the qualitative detection of cows' milk in cheese of different species. However, quantitative results in adulteration control have to be understood as approximate values.

Keywords: species identification, cheese, electrophoresis, HPLC, PCR

EFFECT OF THE USE OF THREE DIFFERENT LAMB PASTE RENNETS ON LIPOLYSIS OF THE PDO PECORINO ROMANO CHEESE

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Two homemade and one industrial lamb paste rennets were used to produce of PDO Pecorino Romano cheese. The homemade rennets came from only suckling (A) or suckling and allowed to graze (B) 30 day-old lambs, slaughtered 6 h after suckling. The third rennet (C) was available commercially. The aim of the work was to investigate the enzymatic differences of the rennets and to study their influence on lipolysis in PDO Pecorino Romano cheese.

Chymosin activity was higher in the homemade rennets (88% and 83% of total clotting activity respectively for rennets A and B) than in the commercial rennet (64%). Both rennet A and B also had higher lipolytic activity (5.60 and 6.17 LFU/g of rennet respectively) than did rennet C (1.78 LFU/g of rennet).

Fifteen cheesemaking trials of PDO Pecorino Romano cheese were carried out in a pilot plant, five replicates for each rennet. Cheese produced with rennet B showed greater lipolysis and different amounts of free fatty acids. Total free fatty acids content was significantly different ($P < 0.001$) in the cheeses (B>A>C). Short chain free fatty acid content showed a similar trend. The level of medium and long chain free fatty acids was very close in A and B cheeses, but was significantly lower ($P < 0.001$) in C cheese.

The percentage of short chain free fatty acids to total fatty acids was higher in the cheese produced with rennets A and B than in those produced with rennet C ($P < 0.01$), whereas rennet C had the highest percentage of both medium and long chain free fatty acids ($P < 0.01$).

Since the lipolytic activity of the rennet strongly influenced the characteristics of the cheese, both the diet of the lambs and the slaughtering conditions should be controlled in order to produce a rennet which can preserve the traditional characteristics of the PDO Pecorino Romano cheese.

Keywords: lamb paste rennet, PDO Pecorino Romano, lipolysis

CHARACTERISATION OF SICILIAN CHEESES BY FLAVOUR ANALYSIS USING SPME-GC/MS

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The flavour composition is an important marker for cheese characterisation; the contribution of short and medium free fatty acids is determining; other substances such as alcohols, chetones, aldehydes, etc. are important, too.

Cheeses possess highly characteristic aromas, indicating the presence of various components mainly dependent, as it is known, on the milk used, production technology, etc. Several researchers studied the flavour of cheeses; unfortunately, they used solvent extraction, which needs long time for the sample preparation, gives modification of the components present, leads to artefacts and disagreeing results for the same type of cheese.

The flavour of the samples analysed were extracted by SPME, a new easy extraction technique, and analysed by HRGC/MS; typical chromatograms for each cheese analysed were obtained (Fig.1). The method, here proposed, provided simple procedure for solvent free sample preparation and no sample manipulation is required; it is quite cheap, moreover, it needs only three hours for the flavour extraction and analysis.

The analyses were carried out on typical Sicilian cheeses such as Provola dei Nebrodi (plastic curd cheese) and Pecorino (sheep milk cheese).

Each cheese will be divided in two samples and each sample, in order to prove method repeatability will be analysed three times. The absolute peak area obtained for the same components determined during the three different analyses were tabulated and the RSD was calculated. Fifty components were identified in the cheese analysed (free fatty acids, esters, alcohols, chetones, etc.) by spectral data, linear retention indices, injection of standards and literature data.

The Provola dei Nebrodi samples were collected over one year and, the flavour composition was studied from the manufacturing and up to 90 days of ripening.

Finally, the identified marker, or the typical chromatogram obtained (Fig.1) were utilised to characterise each Sicilian cheese; differences were observed, moreover, during the ripening.

Keywords: Sicilian cheeses, flavour composition, characterisation, SPME-GC/MS

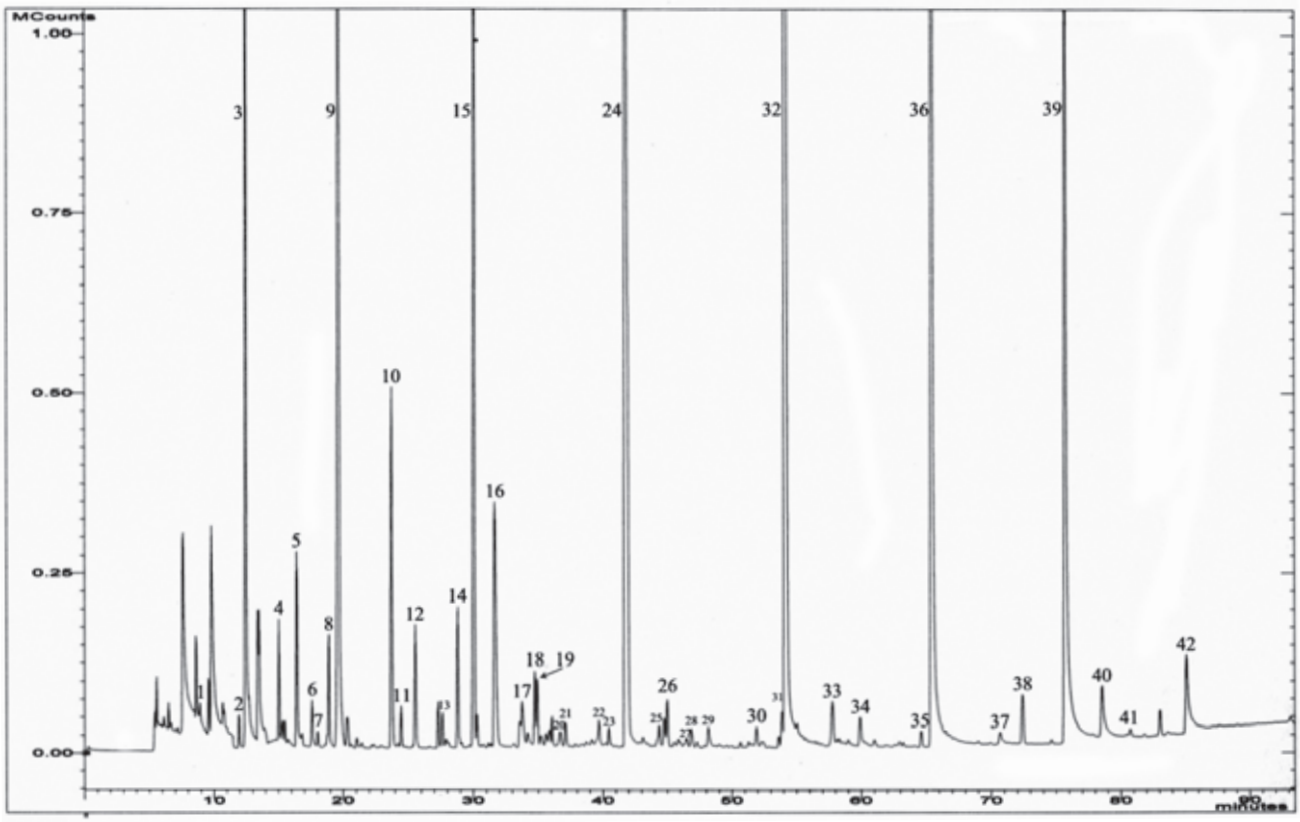

Figure 1 – SPME-HRGC /MS chromatogram of a sample of Provola dei Nebrodi

MORE THAN MOUSTRAP – MEASURING THE SENSORY PROFILE OF CHEESE

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Natural products that ripen over an extended period like cheese and wine pose a difficult problem to the sensory scientist. At best, a single examination of the cheese will provide a snapshot and does not comment on the time course of the ripening product. To establish a comprehensive and balanced view of the cheese, it is essential to determine the sensory profile at different stages of maturation.

There are four essential elements of sensory profiling and these are independent of the product to be assessed:

1. the measuring instrument, i.e. the panel of assessors;
2. the properties to be measured – the sensory vocabulary;
3. the measuring protocol, i.e. the method of measurement;
4. the results, i.e. interpretation of the data in a user-friendly way.

The relative importance of these four pillars of sensory profiling will be illustrated by examples based on cheese. Particular attention will be paid to ensuring the stability of the sensory panel over an extended period of assessment.

Keywords: sensory vocabulary, assessors

THE INFLUENCE OF THE FAT PHASE ON SENSORY CHARACTERISTICS IN A CHEESE IMITATION

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The type and amount of fat and protein, together with the size distribution of the fat droplets in the milk used in cheese production, will influence the microstructure of the final product. In addition, also the sensory characteristics of the cheese will be changed. Since the sensory characteristics of a food product are important, the control of them is of interest.

In this work the type of vegetable fat used, the protein blend, and the homogenising pressure in the production of the imitation milk used were varied and sensory characteristics evaluated. The different vegetable fats have a similar fatty acid composition but differ in solid fat content as well as in crystallisation behaviour. The factors were varied according to an experimental design while the sensory attributes were analysed by a trained sensory panel. In addition, the cheese microstructure was analysed with different techniques and at different magnifications using light microscopy (LM), confocal light scanning microscopy (CLSM), and transmission electron microscopy (TEM). All analyses were performed after an appropriate ripening.

The results show a correlation between the factors and the sensory characteristics, the most important factors being the type of fat and the protein blend. A careful choice of vegetable fat for cheese production can improve the sensory characteristics of the product. Also the impact of the protein blend on the texture of the cheese can be counteracted by the choice of fat.

Keywords: vegetable cheese, sensory analysis, microstructure, microscopy

DEFINING U.S. CHEDDAR CHEESE FLAVOR USING AN ANCHORED SENSORY LANGUAGE

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Cheese flavor represents a crucial aspect for cheese acceptability for direct consumption or ingredient applications. Descriptive sensory analysis of cheese flavor using a defined and anchored sensory language provides a powerful tool for research and marketing communication. Recent work in the U.S. has resulted in a defined and anchored descriptive sensory language for Cheddar cheese flavor. The language has been applied nationally and internationally to define and describe Cheddar cheese flavor. Studies have shown that regional differences in flavor exist in many European cheeses. The objectives of this study were to determine if regional differences exist in flavor of U.S. Cheddar cheeses.

Cheddar cheeses were collected as industrial 18 kg blocks three times per year for two years. Cheeses were received by overnight carrier less than 1 month after production and subsequently aged at 7 °C. Twelve companies representing nineteen industrial locations in four regions of the U.S. participated in the study. Cheeses were aged for 6, 9, 12 and 18 months. At each timepoint, cheese flavor was characterized by descriptive sensory analysis using the defined and anchored Cheddar cheese flavor language. Trained panelists (n=15) evaluated each cheese in duplicate using appropriate descriptive sensory analysis techniques. Data were analyzed using both univariate and multivariate statistical analysis.

Cheddar cheeses were diverse in flavor profile throughout aging ($p<0.05$). Principal component and cluster analysis revealed some flavor similarities throughout cheese aging for locations in the Northeastern United States. Other regions did not have specific consistent flavor similarities and specific flavor profiles were instead associated with particular companies.

Keywords: Cheddar cheese, cheese flavor, regional flavor, sensory analysis

CHANGES OF SENSORY CHARACTERISTICS DURING RIPENING OF THE BLUE CHEESE NIVA

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Blue cheese samples were prepared from cow's milk, and were ripened in presence of *Penicillium roqueforti* mould. A set of 82 samples was tested by sensory analysis using 28 sensory characteristics; both samples of Niva and Niva mixed with butter were examined. The sensory analysis was carried out according to the international standards ISO. Six characteristics of appearance possessed the best reproducible properties, followed by textural data (four characteristics). The texture determined by the sensory analysis correlated with data obtained using Instron apparatus. Direct flavour assessment of Niva (eight descriptors) was more precise than that of cheese samples of Niva ground with butter (ten characteristics). The overall flavour quality and estimation of cheese ripeness were used as the only hedonic descriptors. The optimum flavour and texture quality preceded the full ripeness, which is typical for Czech consumers, also in other brands of imported cheese. Sensory characteristics were found closer to the overall quality than chemical criteria. Relations between sensory characteristics were mostly linear, but sometimes semilogarithmic or double logarithmic, however, close to linear. The multivariate analysis, such as the principal component analysis, canonical analysis, cluster analysis or similar methods, was a good indicator of changes occurring during the ripening, and the results obtained correlated well with the degree of ripeness and the flavour quality.

Keywords: Sensory, ripening, Niva, blue cheese

EVALUATION OF CHEESE QUALITY AND RIPENING CHARACTERISTIC BY SPECTROSCOPY AND CHEMOMETRICS

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Quantitative Near Infrared (NIR) and Mid Infrared (MID) spectrometry are established techniques in the dairy industry. Both methods have proved to be excellent in quality control mainly due to precision and to the fact that no or limited sample preparation is necessary, hence the analysis is very simple and fast. These qualities have made NIR and MID superior techniques in in-, on- or at-line monitoring and control.

Compared to NIR and MIR fluorescence spectrometry is a less widespread method, primarily used in research laboratories. But recently the use of this technique has been reported in a number of non-trivial applications in cheese.

As a consequence of the complexity of the spectra, multivariate mathematics (chemometrics) is normally applied in order to extract useful information.

For example a set of spectra can be analysed by Principal Component Analysis (PCA) to find the main phenomena in the set, or the spectra, in combination with one or more quality values, can be used to build predictive Partial Least Squares (PLS) regression models.

But also modern multi-way chemometrics, e.g. Parallel Factor Analysis (PARAFAC) has been applied in studies of cheese quality.

The concentrations of constituents such as water, protein, fat and carbohydrate can be determined using classical methods for calibration and validation, but food properties (e.g. textural and flavour) can also be monitored on a continuous basis.

Protein breakdown has an obvious role in determining the texture and background flavour in all matured cheese varieties. Proteolysis in cheese is usually described by changes in peptide patterns (analysed e.g. by reversed phase liquid chromatography). It is demonstrated that latent structures in data from peptide analysis can be predicted from spectroscopy data.

Keywords: Infrared, Fluorescence, Chemometrics, Cheese

APPLICATION OF FT-NIR AND FT-IR SPECTROSCOPY TO STUDY THE SHELF-LIFE OF CRESCENZA CHEESE

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Crescenza is a fresh cheese in which structural and chemical modifications can occur during the shelf-life, due to intense enzymatic activity. The severity of these modifications is related to the transport and storage conditions, as determined through measurements of colorimetric and structural variations of the cheese paste, and the development of aromatic compounds influencing sensory evaluation. Crescenza „freshness" is generally associated with low acidity, limited proteolysis, and no bitter taste.

In this study, the FT-NIR and FT-IR spectroscopy was applied in order to evaluate the shelf-life period to maintain Crescenza „freshness". Two different types of Crescenza cheese, stored at 10 ±1°C for the declared shelf-life period (20 days), were analysed at different times.

Spectral data were collected in two spectral ranges, from 12000 cm^{-1} to 4400 cm^{-1}, by using a FT-NIR spectrometer with an optic fibre working in diffuse reflectance, and from 4000 cm^{-1} to 700 cm^{-1}, by using a FT-IR spectrometer with an ATR cell. Measurements were carried out in replicates using the original packets. A qualitative statistical software was used for data processing.

Chemometrics applied to FT-NIR and FT-IR spectroscopy was able to detect the decrease of Crescenza „freshness" and to determine a critical day during the shelf-life period. These results are in agreement with those reported both in previous studies and in physical-chemical and chemical tests, where different analitycal techniques were applied. The principal absorption bands involved were determined.

Physical, chemical, and microbiological factors affect the product „freshness" having some negative influence on the peculiarity of cheese from production to consumption. Therefore, the main advantage of using the IR spectroscopic techniques is to rapidly draw a profile of the product related to its total quality.

Keywords: spectroscopy, chemometrics, shelf-life, Crescenza cheese

PREDICTION OF QUALITY PARAMETERS AND AGE IN CHEDDAR-TYPE CHEESE BY NEAR INFRARED REFLECTANCE SPECTROSCOPY AND MULTIVARIATE DATA ANALYSIS

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Cheese quality assessment includes chemical and sensory testing, procedures unsuitable for routine use. Near infrared (NIR) spectroscopy is a rapid, easy-to-use, and non-destructive analytical technique. Little research on its use for prediction of sensory characteristics or age of cheese has been published.

Cheddar cheese samples were manufactured and aged for 9 months at 4 °C. At time periods of 1, 2, 4, 6 and 9 months, aliquots were subject to sensory and NIR spectroscopic analysis. Calibrations for a number of sensory parameters and age were developed using partial least squares (PLS) regression against spectral data using a number of wavelength ranges and data pre-treatments. Best models used spectral data between 750 and 1098 nm in all cases.

Textural attributes „rubbery" and „crumbly" were modelled well. Rubbery texture (range = 6.4–34.9) was predicted with a standard error of cross-validation (SECV) of 3.8 and a correlation coefficient (R) of 0.76 (6 PLS loadings). Crumbly texture (range 1.6–20.9 units) was predicted best by an 8 loading model (SECV = 2.2 units; R = 0.87).

Age was best predicted using spectral data pre-treated by scatter correction and a 2nd derivative. A SECV of 0.90 months was obtained with a correlation coefficient of 0.95 (8 loadings).

These results suggest a role for NIR spectroscopy in the off-line quality assessment of Cheddar cheese.

Keywords: Cheddar cheese; near infrared spectroscopy; chemometrics

ELECTRONIC NOSE: NEW TOOL IN MODELLING THE RIPENING OF DANISH BLUE CHEESE

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Objective aroma assessments for monitoring the ripening process of Danish blue cheese are of great interest in the industry. Instrumental techniques and sensory panels are generally characterised by increased capital cost and require skilful personnel. An E-nose system might offer a number of advantages such as less skills required, low capital cost, automated artificial intelligence etc.

Danish blue-veined cheese is a traditional semi-soft cheese produced from heat-treated cow's milk. Ripening of this type of cheese depends on complex microbial interactions involving the primary lactic starter culture, the secondary starter culture, *Penicillium roqueforti,* mould contaminants and yeast.

It is well established that during the ripening process of Danish blue cheese the growth of *P. roqueforti* contributes extensively to the unique flavour and texture of Danish blue cheese. The characteristic flavour and taste of blue cheese are closely connected to the ripening stage and can be directly related to the changes in appearance, to aroma formation due to lipid degradation and oxidation and to texture changes due to proteolysis.

The possibility of implementing an electronic nose system in order to evaluate the ripening stage of Danish blue cheeses (5 to 12 weeks after brining) was investigated and results were considered very promising.

In this experiment electronic nose technology has been applied directly on representative cheese samples in order to get an objective characteristic of the cheese aroma profile at the different stages of ripening. These results were evaluated and compared to sensorial analysis performed by a trained panel and aroma analysis examined by means of sampling by diffusion from the headspace of the cheese and gas chromatography-mass spectrometry (GC-MS) carried out on the same cheeses.

Keywords: Danish blue cheese, ripening, e-nose, GC-MS, sensory

INVESTIGATION AT THE MOLECULAR LEVEL OF SOFT CHEESES QUALITY AND RIPENING BY INFRARED AND FLUORESCENCE SPECTROSCOPIES AND CHEMOMETRICS – RELATIONS WITH RHEOLOGY PROPERTIES

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Classical techniques such as chemistry, biochemistry and rheology are usually used for the characterisation of cheeses. Recently, new investigations have been successfully attempted by coupling infrared and fluorescence spectroscopies. Chemometric methods such as common components and specific weights analysis (CCSWA) have been recently used to 1) describe in a simple and synthetic manner the overall information collected, 2) extract and use the relevant information related to the structural changes of the studied component, 3) provide tools for a molecular interpretation of the results and 4) investigate the relations between structures and rheology properties.

Experimental soft cheeses, i.e., 12 traditional and 12 stabilised, made using typical making schemes were manufactured at the INRA pilot-scale dairy-plant. The gross chemical compositions of the cheeses were made according to an experimental design: the dry matters and the fat/dry matters of the stabilised soft cheeses were fixed to 44% & 48%, and 51% & 55%, respectively, whereas the values for traditional soft cheeses were 38% & 42%, and 46% & 50%, respectively. Cheese physico-chemical parameters were determined. Spectra were recorded directly on samples. The emission spectra of tryptophan residues (305-400 nm) were recorded with the excitation wavelength set at 290 nm and the excitation spectra of vitamin A (250–350 nm) were recorded with the emission wavelength set at 410 nm. Infrared spectra were recorded between 3000 and 900 cm^{-1} at a resolution of 4 cm^{-1}. The rheology method used was the uniaxial compression test at constant displacement rate. Cheese samples were analysed before salting and after 30 days of ripening by the different methods mentioned above. CCSWA was applied to the seven data sets.

The results showed that the common component 1 discriminating young and ripened cheeses explained 95%, 91%, 73.6% and 51% of the inertia of 900–1500 cm^{-1} infrared region, 2800–3000 cm^{-1} infrared region, 1500-1700 cm^{-1} infrared region and rheology data, respectively. It was also shown that common component 3 discriminating cheeses as a function of the technology explained 54%, 26.4% and 11% of the inertia of vitamin A spectra, chemical data and rheology data, respectively. The spectral patterns associated with the common components allowed molecular interpretations of the discrimination.

Keywords: soft cheese, fluorescence, infrared, chemometric

CHARACTERISATION OF CHEESE VARIETY AND MATURITY ON THE BASIS OF PROTEOLYSIS

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The main features of an acceptable cheese are desirable texture and flavour in the context of a particular cheese type or variety. These features can be assessed by trained sensory panels and there is interest in using instrumental methods because there can be inter-panel differences in sensory assessment. Both flavour and texture are considered to be strongly influenced by the extent and type of proteolysis that has occurred in the particular cheese under examination. In the past, methods such as the proportion of the protein that is water-soluble, alcohol-soluble or soluble in dilute trichloroacetic acid have been used to examine proteolysis. Alternatives such as polyacrylamide gel electrophoresis in a urea buffer, size-exclusion chromatography or reverse phase high performance liquid chromatography (RP-HPLC) have also been used. RP-HPLC methods can readily resolve up to 100 peaks and some peaks contain several different peptides. Application of multivariate statistical methods enables the differentiation of a group of cheese types into different categories on the basis of the changes in the intensities of the RP-HPLC or SE-HPLC peaks. However, when these techniques are used to analyse a diverse range of New Zealand cheese of one type, maturity trends are evident but there is considerable overlap between cheese from different manufacturers and differentiation on the basis of manufacturer is not possible. The data can be used to develop maturity indices.

Keywords: characterisation, maturity, proteolysis

MONITORING OF PROTEOLYSIS PHENOMENA USING ANTIBODIES SPECIFICALLY DIRECTED AGAINST THE ENZYME CLEAVAGE SITE ON ITS SUBSTRATE

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Proteolysis is a generic biochemical process that plays a major role in most of the biological activities. Proteolysis can be monitored by direct quantification of the protease activity, using specific substrates that, when hydrolyzed by the enzyme, will produce an absorbance, a fluorescent or a luminescent signal. Other techniques consist in the monitoring of the appearance of some peptides, known as markers of the enzymatic activity. Finally, the hydrolysis of peptide bonds can be directly studied using high technology methodologies, such as atomic force microscopy.

An alternative strategy for monitoring this biochemical process is proposed here. This approach is based on the production of rabbit polyclonal antibodies against synthetic peptides mimicking the cleavage site on the substrate of the enzyme responsible for proteolysis. As long as the molecule's cleavage site is intact, the antibody will bind to the protein. However, after cleavage of the peptide bond by the protease, the antibody will no longer be able to recognize the substrate.

Hydrolysis of β-casein by plasmin, the main indigenous protease of milk, was chosen as a model for this study. Six synthetic peptides which included or bordered the plasmin-sensitive bonds Lys_{28}-Lys_{29}, Lys_{48}-$Ileu_{49}$, Lys_{105}-His_{106} and Lys_{107}-Glu_{108} were produced and administered to rabbits. Five out of 6 antisera cross-reacted with β-casein and were used in five indirect ELISAs. Hydrolysis of sites Lys_{28}-Lys_{29}, Lys_{48}-$Ileu_{49}$, Lys_{105}-His_{106} and Lys_{107}-Glu_{108} was monitored with these ELISAs throughout the ripening of Swiss-type cheese allowing to determine which sites are preferentially cleaved during cheese maturation.

Keywords: antibody, ELISA, plasmin, proteolysis

APPLICATIONS OF HIGH PRESSURE PROCESSING IN CHEESE MANUFACTURE AND RIPENING

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High pressure (HP) processing is a recently-introduced food processing technology, with many applications for food products including fruit juices and shellfish. It may also have applications either in treatment of milk prior to cheesemaking, or in treatment of cheese directly, as reported herein. HP treatment of Cheddar cheese at 50 MPa for 3 d accelerated ripening through increased proteolysis of α_{s1}-casein, particularly for very young (< 1 week-old) cheese; however, the magnitude of such acceleration was small. Manipulation of processing conditions (pressure and duration of treatment) increased the degree of acceleration, but the overall effect remained quite small. HP treatment of immature Mozzarella cheese significantly affected its functional properties, accelerating development of desirable cheese characteristics for applications such as pizza toppings. The magnitude of the effect of HP on Mozzarella cheese was somewhat variable, and not all factors influencing this variability are apparent. Of ripening enzymes, plasmin was highly resistant to HP-induced inactivation in cheese, while chymosin was inactivated at > 400 MPa. HP treatment of milk for cheesemaking has several interesting effects; growth of starter bacteria is accelerated, and cheese yield may be increased through incorporation of HP-denatured whey proteins without increasing curd moisture content. Overall, the effects of HP treatment on cheese itself are variety-dependent, and may be related to changes in physicochemical conditions or proteolysis; the benefit of HP treatment, relative to the cost of equipment, requires careful consideration. HP treatment of milk for cheesemaking may be of more immediate potential applicability, although further aspects of the effects of HP on cheese milk remain to be clarified.

Keywords: high pressure, cheese, cheesemaking, ripening

RHEOLOGICAL AND CALCIUM EQUILIBRIUM CHANGES DURING RIPENING OF CHEDDAR CHEESE

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Most studies on the ripening of Cheddar cheese have focused on proteolysis and textural properties. It is well known that the Ca concentration influences the textural properties of cheese. We believe that the amount of Ca still within the casein particles of Cheddar cheese, i.e., insoluble Ca, plays a key role in modulating cheese texture and that changes from insoluble to soluble Ca occur during ripening that contribute to textural changes. Standard full-fat Cheddar cheese was manufactured and ripened for up to 9-mo. Rheological properties of cheese were determined using dynamic low-amplitude oscillatory rheology over the temperature range 5 to 80 °C. Changes in the Ca equilibrium were determined using acid-base buffering of cheese homogenates or analysis of cheese serum (juice) expressed by hydraulic pressure. Proportion of insoluble Ca as a % of total Ca decreased from ~70 to 50–60% between d 1 and 9-mo; most changes occurred within the first 4 wk. Storage modulus (G') of cheese decreased during heating. G' values at low temperature (e.g. 5 °C) were higher in older cheese (e.g. 9-mo) than young cheese. G' values at high temperature (e.g. 80 °C) decreased rapidly during ripening. At low temperatures (e.g. 5 °C) the loss tangent values of cheeses were similar during ripening. At temperatures > 40 °C the loss tangent increased to reach a maximum at temperatures ~70 °C in young cheese and lower maximum temperatures were observed with ripening. Maximum loss tangent values increased substantially during the first 3 weeks and then slightly decreased or showed little change. The increased loss tangent behaviour at high temperatures in young cheese coincided with increased melt and flow. These results indicate that Ca equilibrium changes also contribute to the initial changes in cheese texture since they occur concomitantly with well known initial proteolytic events.

Keywords: rheology, calcium, melt, cheese ripening.

BIOCHEMISTRY OF CHEESE FLAVOR DEVELOPMENT: NEW INSIGHTS FROM THE GENETICS AND PHYSIOLOGY OF LACTIC ACID BACTERIA

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Microorganisms and enzymes (endogenous, added, and microbial) in the cheese matrix gradually convert milk carbohydrates, citrate, proteins, and lipids in a manner that ultimately yields the desired final product. Lactic acid bacteria (LAB) were linked to cheese flavor development more than 100 years ago, however the variation and complexity in the microbiota and enzyme content of cheese has hindered efforts to evaluate the relative contributions of specific microorganisms and enzymes. Although many details regarding the contribution of LAB to flavor must still be elucidated, current knowledge indicates that LAB influence cheese flavor development via several key mechanisms. These include: 1) hydrolysis of proteins into peptides and free amino acids; 2) metabolism of free amino acids; and 3) hydrolysis of milk lipids into free fatty acids and their conversion to esters.

This presentation will include both an overview of the biochemistry of LAB's contribution to cheese flavor and our recent genetics-based approach for understanding how *Lactobacillus helveticus* CNRZ32 influences cheese flavor development. We recently assembled a draft-quality (4X) genome sequence of *L. helveticus* CNRZ32. Genomic analysis identified genes encoding numerous additional proteolytic enzymes, including proteinases, endopeptidases, aminopeptidases, dipeptidases, and tripeptidases. Also, numerous genes encoding putative amino acid catabolic pathways were identified. Of particular interest due to their potential role in debittering bacterial-ripened cheeses are the three additional endopeptidases identified in the draft genomic sequence. The genes encoding these three endopeptidases, designated *pepO3*, *pepF* and *pepE2*, have been characterized and their role in the hydrolysis of model bitter peptides evaluated. Currently, we are utilizing DNA microarrays to characterize expression of the proteolytic and amino acid catabolic systems of *L. helveticus* CNRZ32.

Keywords: lactic acid bacteria, genetics, physiology, cheese flavor

CHARACTERISATION OF A DECARBOXYLASE INVOLVED IN THE FORMATION OF THE POTENT FLAVOUR COMPONENT 3-METHYLBUTANAL

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Flavour is one of the most important attributes of cheese. The breakdown of milk components during ripening gives rise to a series of volatile and non-volatile compounds which contribute to cheese flavour. The enzymatic degradation of amino acids by, starter and non-starter, micro-organisms is an important step in the production of volatile flavour compounds, such as sulphur compounds and compounds derived from branched-chain and aromatic amino acids.

In our study, various micro organisms, used in cheese making, were analysed for their potential to convert leucine into flavour components, most notably 3-methylbutanal. A large variation between, but also within, species was observed for various enzyme activities which are involved in leucine conversion. In particular, α-keto acid decarboxylase activity, leading to 3-methylbutanal, was found to be present in only few of the strains tested. It is proposed that this activity is rate controlling in the leucine conversion pathway leading to the flavour compound 3-methylbutanal.

Current research is focussed on the purification and characterisation of α-keto acid decarboxylase enzymes. Based on homology studies, several decarboxylase genes have been identified in *L. lactis* genomes. Their overexpression, however, generally does not result in enhanced 3-methylbutanal production. A gene coding for a α-keto acid decarboxylating enzyme recently has been identified in *L. lactis* ssp. *cremoris* B1157, using a fast method for screening of knock-out mutants. After construction of an overproducing strain high levels of production of 3-methylbutanal were measured.

Detailed knowledge of the α-keto acid decarboxylating enzyme and of other essential enzyme activities needed for flavour formation, is important for selection of new starter cultures. Such new cultures will provide novel possibilities regarding cheese flavour diversification and accelerated cheese ripening.

Keywords: flavour, leucine, decarboxylation, 3-methylbutanal

PATHWAYS FOR α-KETOGLUTARATE FORMATION IN LACTIC ACID BACTERIA AND THEIR ROLE IN AMINO ACID CATABOLISM

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In lactic acid bacteria (LAB), the production of α-ketoglutarate (α-KG), required as amino group acceptor in the amino acid transamination, is often a limiting factor for the conversion of amino acids to aroma compounds in cheese. The presence of an α-KG formation pathway from components present in cheese or milk (citrate, glutamate, pyruvate) appears thus essential for aroma formation from amino acids. Theorically, three main α-KG formation pathways exist in bacteria. The first utilises glutamate and is catalysed by a glutamate dehydrogenase (GDH). The second pathway utilises citrate or pyruvate and glutamate, and necessitates citrate lyase or pyruvate carboxylase and aspartate transaminase. Finally, the third pathway also utilises citrate and requires the action of aconitase (ACN) and isocitrate dehydrogenase (IDH). The utilisation of citrate from the medium also requires the presence of a citrate permease.

The objective of the study was to search for and select LAB strains exhibiting the enzymatic activities necessary for each of these pathways, in order to evaluate their impact on amino acid catabolism.

For the first pathway, we detected a weak NADP-dependent GDH activity in several LAB species, while a NAD-dependent GDH activity was only observed in a few strains of *S. thermophilus* and *L. lactis*. *L. lactis* subsp. *diacetylactis* possesses all the activities required for the second pathway. Finally none of the tested LAB strains exhibited the activities necessary for the third one. Only those strains exhibiting the activities necessary for the first or the second α-KG production pathway catabolised amino acids in a medium containing citrate and/or glutamate. These results show that functional pathways for α-KG production exist in LAB and the presence of such a pathway is a major criteria for the selection of strains capable of catabolising amino acids. However it remains to verify if the selected strains can produce aroma compounds in cheese.

Keywords: amino acid catabolism, lactic acid bacteria, α-ketoglutarate, aroma compounds

ESTERASES OF LACTIC ACID BACTERIA AND CHEESE FLAVOUR

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Esterases of lactic acid bacteria (LAB) are a diverse collection of enzymes grouped by their hydrolytic activity on chromogenic ester substrates. Often this activity bears no relation to the true nature of the enzyme activity and has little or no relevance to the impact of the enzyme on milk fat substrates. There are two ways in which esterases might impact on flavour generation from milk fat substrates in cheese: hydrolysis of milk fat glycerides to release free fatty acids, and synthesis of ester flavour compounds. To explore the potential for esterases of LAB to impact on flavour development during cheese ripening, we have compared esterases from *Lactococcus lactis, Streptococcus thermophilus* and *Lactobacillus rhamnosus* in a number of different reaction systems, ranging from *in vitro* assays to experimental cheese systems.

The hydrolase potential of esterases is the best understood activity of LAB esterases, because this is the marker activity for the enzyme group. Tributyrin esterase (estA) from *Lactococcus* is most active on monoacylglycerols, and shows some activity on diacylglycerols and tributyrin substrates. The enzyme shows a clear preference for glyceryl esters of short chain fatty acids. An esterase from *Streptococcus thermophilus* also has peptidase activity and, based on gene sequence data, is identifed as X-prolyldipeptidyl aminopeptidase (pepX). This illustrates the plurality of enzyme activity that can occur with esterases. The major esterase (estAA7) from *Lactobacillus rhamnosus* HN001 appears to be yet another type of esterase.

The ability of esterases to synthesise short chain ester compounds is less well understood. Ester synthesis in cheese and other dairy systems has long been regarded as a 2-step process in which free fatty acids are released by hydrolysis of milk fat and esterification of free fatty acids with an alcohol then follows. However, we have shown that esterase enzymes of a number of lactic acid bacteria form flavourful esters in a single-step transesterification reaction in which ethanol is the acceptor. Tributyrin esterase from *Lactococcus* and esterases from *Streptococcus* produce significant amounts of ethyl esters when glyceride substrates are presented together with ethanol.

We are now able to direct cheese flavour development by directing esterase activity in cheese through control of milk fat substrates, the cheese environment, and esterase levels.

Keywords: esterase, fatty acids, esters, LAB

COMPARISON OF PURGE AND TRAP AND SOLID PHASE MICRO EXTRACTION TECHNIQUES FOR THE STUDY OF VOLATILE ORGANIC COMPOUNDS IN THREE PDO EUROPEAN CHEESES

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Eighteen loaves of three PDO cheeses (six loaves of each), namely Ragusano (Italy), Manchego (Spain) and Gruyère (Switzerland) were studied for volatile organic compounds (VOC) using Solid Phase Micro Extraction (SPME) and Purge and Trap (P&T) extraction, coupled to Gas Chromatography-Mass Spectrometry/Flame Ionisation Detection/ Olfactometry (GC-MS/FID/O).

SPME was carried out with a three-phase StableFlex fibre (Divinyl benzene/Carboxen/Poli dimethyl siloxane). P&T extraction was performed with a Tekmar 2100™ equipped with a tenax trap.

Gas Chromatography was performed in an HP 5890 GC equipped with a DB-Wax column, with simultaneous FID and MS detection. The same GC apparatus was used for the olfactometric detection, by exchanging the line from the FID to the olfactometric port.

In total, 102 and 84 VOC were found by using SPME and P&T respectively. Differences were significant for the abundances of most VOC between the three different cheese varieties using the same extraction method. Differences between the extraction methods were also significant for the abundances of most VOC within the same cheese variety.

SPME showed a better extraction efficiency for volatile fatty acids, aromatic compounds and, in general, for the higher molecular weight (MW) methyl ketones and esters. P & T showed a better extraction efficiency for hydrocarbons, alcohols, diacetyl and, in general, for the lower MW methyl ketones and esters.

Using SPME, Gruyère cheeses revealed higher abundances of 2,6-dimethyl pyrazine, n-alkanals and branched chain acids compared to the two other cheese types. Manchego cheeses showed higher abundances of 2-methyl ketones and aromatic compounds. Ragusano cheeses showed higher abundances of ethyl esters and linear fatty acids.

Using the P&T method, Gruyère cheeses showed higher abundances of alkenes, diacetyl and branched chain alkenols compared to the other cheese varieties. Manchego cheeses revealed higher abundances of alkanes, other alcohols, low MW ketones and propyl esters. Ragusano cheeses again showed higher abundances of ethyl esters, butyl esters and linear fatty acids.

By GC-O the three cheese varieties studied revealed potent aroma compounds, such as diacetyl (buttery odour), esters (fruity notes), methional (potato-like odour), butyric acid (cheesy odour), and 3-methyl-1-butyric acid (cheesy odour).

Keywords: Purge and Trap, Solid Phase Micro Extraction, Volatile Organic Compounds, PDO European Cheese

CULTURES FOR THE RIPENING OF SMEAR CHEESES

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During the past five years several projects at the Federal Dairy Research Centre, Kiel, have been dedicated to the development of surface cultures for smear cheeses. The typical surface microflora of semi-soft cheeses (Tilsit-type), soft cheeses (Limburg-, Romadour-type) and acid curd cheeses (Harzer Roller-type) was determined. The minimal composition of the surface starter cultures which allows rapid smear development was determined for these varieties.

Cultures for semi-soft cheeses should contain *Debaryomyces hansenii, Staphylococcus equorum, Corynebacterium casei, Microbacterium gubbeenense* (or *Arthrobacter nicotianae*), and *Brevibacterium linens*. Yeasts and staphylococci can be inoculated into the cheese brines at a level of 10^4–10^6 cfu/ml instead of smearing them on the cheese surface. The smear should contain the other bacteria at a concentration of at least 10^7 cfu/ml for each species which allows fast deacidification of the cheese surface and smear development. After seven days, the surface pH should be >pH 7 and the smear $>10^9$ cfu/cm^2. Yeasts and staphylococci reach maximum numbers in the first week, *Microbacterium* or *Arthrobacter* in the second week. The percentage of *B. linens* in the total cell counts is usually low, with a maximum reached after 2–4 weeks of ripening. Highest cell counts in all stages of ripening are always observed for corynebacteria.

Surface cultures for soft cheeses should contain *Geotrichum candidum* which is responsible for the typical white streaks and patches on the orange coloured cheeses and for typical aroma development. *G. candidum* should be inoculated into the cheese milk at 10^2 cfu/ml. The presence of *Corynebacterium casei* is not essential for typical soft cheese ripening. The use of *S. equorum*, not regularly found on the surface of commercial soft cheeses, accelerates deacidification and smear development without detectable influence on aroma development. A high percentage or *Microbacterium* or *Arthrobacter* is usually found (near 100% for some cheeses) with no or low counts of *B. linens*. For soft cheeses, it can be assumed that interactions of *Microbacterium* or *Arthrobacter* with *G. candidum* are responsible for typical aroma development.

The ripening of acid-curd cheeses is completely different because they are produced from quarg (dry mass >30%). Two yeasts, *Kluyveromyces marxianus* and *Candida krusei*, should be inoculated into the milk used for quarg production. The quarg should be stored for at least five days at about 15°C to allow the development of the yeast flora. Staphylococci seem to be essential for cheese ripening. *S. equorum* can be added to the quarg with ripening salts to replace the non food-grade *S. saprophyticus* which is always present on acid-curd cheeses. Suitable corynebacteria for spraying of cheeses are *Brevibacterium linens* and *Corynebacterium variabile*.

Smear cheese ripening with defined surface cultures without the typical old-young-smearing was used successfully many times on laboratory scale. At present, defined cultures have been tested on a pilot scale. The introduction of defined cultures could help to reduce the otherwise inevitable levels of contaminating enterobacteria, enterococci, saprophytic staphylococci, moulds and pathogens.

Keywords: food safety, smear cheeses, surface cultures, Corynebacterium, Brevibacterium, Microbacterium, Arthrobacter

DYNAMICS OF THE SURFACE MICROFLORA OF BACTERIAL SMEAR-RIPENED TILSIT CHEESE DETERMINED BY T-RFLP ANALYSIS

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The surface microflora of smear cheeses strongly influences the appearance, flavour and texture, as well as the ripening time of the cheese. The composition of the microflora of three Tilsit- type smear-ripened cheeses was studied after one, two, four and eight weeks of ripening. Cheese was treated with an undefined smear starter mix or with defined smear starter mixes. Defined mixes comprised strains of *Microbacterium barkeri*, *Corynebacterium casei*, *Corynebacterium variabilis, Staphylococcus sciuri*, *Staphylococcus equorum* and *Brevibacterium linens.*

The microflora on the cheese surface at each time point was analyzed using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis of the partial small subunit ribosomal gene.

Starter strains were present throughout ripening. Additional strains, apparently originating from the environment, were found. Maximal diversity was observed between weeks two and four, followed by a decrease towards the end of ripening. Most of the strains reached a maximum numbers in week four and had almost disappeared after eight weeks. However, the *Corynebacterium* species tended to increase in relative numbers rapidly towards the end and were the dominant bacterial genus on the surface of the fully ripened cheese. Observations were in agreement with those from culture-based studies.

T-RFLP-analysis was found to be an excellent tool for the rapid and specific assessment of population composition and dynamics that facilitates the reconstruction of starter mixes.

Keywords: smear cultures, T-RFLP analysis, population dynamics, cheese

NEW TRENDS IN MICROBIAL FORMULATION OF SMEAR CHEESES FOR FLAVOUR AND COLOUR GENERATION

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The microbial mixtures found in soft cheeses are particularly complex. Their addition to milk or to the rind washing solutions should lead to the generation of various properties, such as the limitation of undesirable flora, texture, colour and flavour generation.

To understand the generation of these desired functionalities, it seemed reasonable to identify the biological activities used, by the different species (strains) present on the cheese surface, to produce the desired functions. This screening step can be done on a model curd medium quite efficiently for the yeasts and for the bacteria.

There is at the moment no method other than the empirical expertise for the formulation of the microbial mixtures able to produce the desired functionalities. Which rule could help to combine them? Some examples, such as the combination of *Geotrichum candidum* with *Penicillium camemberti* were quite encouraging, because the various typicalities of the pure cultures lead to mixed cultures on curd with the same variety of typicalities and finally the same was observe in cheeses. However, this approach always failed with bacteria. In associations with different yeasts, the weigh of the yeast flavour was much higher than the one of bacteria. The colour of the bacterial strain was dependant on the yeast used for curd deacidification.

In order to amplify the activity of bacterial strains having having rather strong flavouring properties, they were associated with bacteria having efficient hydrolytic properties. The flavour differences observed in pure cultures were not always observed in mixed cultures on the same biologically deacidified curd medium. Consequently, the success of an approach by addition of flora having specific functions seems to be quite random.

Consequently we changed of approach using the combination of a larger number of bacteria and tried to find subgroup of bacteria able to produce the major properties research. The use of molecular methods (PCR-SSCP) permitted to follow the growth of the main species growing among the whole mixture. The first results of this approach will be discussed.

Keywords: flavour, colour, ecosystems, microbial formulation

YEASTS FOR FLAVOUR AND NUTRITIONAL ENHANCEMENT OF CHEESE

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The objective of this study was to manufacture a cheese with novel flavour and with an elevated level of conjugated linoleic acid (CLA) for enhanced nutrition. Our earlier experiments with Cheddar demonstrated that yeast lipases were active in the cheese and selectively released unsaturated long-chain fatty acids (FFA) from milk fat. Also, three strains of propionibacteria were shown in our laboratory to convert free linoleic acid to CLA. By using yeast lipase and propionibacteria together, we attempted to generate CLA in the ripening cheese.

Two yeasts, one strain of *Geotrichum candidum* and one strain of *Yarrowia lipolytica*, were used as anaerobic adjuncts in a dry-salted, washed-curd cheese. The yeasts were grown under optimum conditions for lipase production. Two non-starter lactic acid bacteria (NSLAB) were used as adjuncts: *Lactobacillus fermentum* to produce ethanol and thus promote ester formation to mask the undesirable flavours of fatty acids, and a strain of *L. rhamnosus* to control the adventitious NSLAB flora. Three strains of *Propionibacterium* were added to three different 375L cheese vats along with starter and the other adjuncts. The resulting blocks of cheese were matured at 20 °C.

The proportions of long-chain unsaturated FFA's (oleic, linoleic and CLA) were higher in the yeast cheeses throughout ripening than in cheese with no added yeast. This indicates that the yeast lipase(s) selectively hydrolysed unsaturated long-chain fatty acids from the milk fat. Higher concentrations of ethyl esters were detected in yeast cheeses. The proportion of total CLA was the same in both control and experimental cheese over the four months ripening period. We conclude that free linoleic acid was produced by the yeast lipase(s) but there was no conversion to CLA by the isomerase of the propionibacteria, probably due to unfavourable environmental conditions in the cheese.

Keywords: Adjunct, CLA, Lipase, Yeast

AROMA DEVELOPMENT IN SEMI-HARD REDUCED-FAT CHEESE INOCULATED WITH *LACTOBACILLUS PARACASEI* STRAINS WITH DIFFERENT AMINOTRANSFERASE PROFILES

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Aminotransferase (AT) activity is the first step in the formation of aroma compounds from amino acids in cheese mediated by lactic acid bacteria. The AT activity and specificity for different amino acids are strain dependent for *Lactococcus*, as well as *Lactobacillus*. The influence on flavour formation in cheese of three *Lb. paracasei* strains (CHCC 2115, 4256, and 5583) with different AT profiles was investigated in cheesemaking trails. All strains had AT activity on Asp and Met, but especially on branched-chain amino acids (BcAA); CHCC 2115 had 5 to 10 times higher BcAA activity than the other strains, and CHCC 5583 had activity mainly on Leu. All strains had activity on aromatic amino acids (ArAA), but the strain with very high BcAA-AT activity did not have higher activity than the other strains against ArAA. Reduced-fat, semi-hard, round-eyed cheese was made from cows' milk using mesophilic starter. Three cheese vats inoculated with starter and a *Lactobacillus* strain, and a control cheese vat inoculated with starter only (4 vats) were made during one day and repeated three times.

The cheese manufacturing procedure was not influenced by the *Lactobacillus* adjunct and similar cheese was made from all vats. Unfortunately, clostridia spores were present and developed slightly in all cheeses but not with higher impact than significant differences in flavour profiles were detected between cheeses made with different *Lactobacillus* strains. In all cheeses made with CHCC 4256, significantly more flavour compounds were produced from BcAA, and they were also preferred for tasting aromatic and sweet with no bitterness compared to the other cheeses. Cheeses with CHCC 4256 also contained considerably more diacetyl and acetoin than the others, and especially the control cheeses were low in those compounds. Diacetyl and acetoin might have been produced from Asp (and Asn).

Keywords: cheese, *Lactobacillus paracasei*, Aminotransferases, aroma formation

BIOCHEMISTRY OF NSLAB IN NON-GROWTH ENVIRONMENTS

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Non-starter lactic acid bacteria (NSLAB) occur in Cheddar cheese and contribute to the development of flavour during extended periods of maturation. This paper describes some of the physiological and metabolic traits of two *Lb. casei* strains that occurred either transiently or more persistently in samples taken from Australian cheddar cheeses over 63 weeks, with emphasis on metabolites formed under starvation conditions.

The two strains studied were considered to be related biotypes on the basis of genetic analysis, including pulse-field gel electrophoresis patterns but could be differentiated on the basis of carbohydrate metabolism profiles (ability to utilise tagatose), survival in low pH environments and protein profiles. In a minimal medium based on „cheese juice", both strains showed requirements for eight amino acids and several vitamins. This medium was used to determine the metabolites formed under different conditions of nutrient supplementation for periods of up to 60 days. Both strains failed to grow in the minimal medium containing all growth requirements if a carbohydrate source was not provided. Trials were conducted on cells in minimal medium containing tryptone plus additional specific growth requirements in the presence of either ample (1%) or growth-limiting (0.2%) levels of lactose. In some trials, specific amino acids were provided at higher concentrations to determine their impact on metabolite formation. Under starvation conditions (0.2% lactose), peptide consumption occurred as determined from changes in HPLC fingerprints, whereas peptide profiles for cells incubated in the presence of higher concentrations of lactose did not change. The range of metabolites seen during lactose starvation also differed and this was particularly noticeable when the medium was supplemented with high concentrations of specific amino acids. The amount of new end products made decreased when lower concentrations of amino acids were supplied, indicating a direct link between the products and the presence of specific amino acids.

Keywords: *Lb. casei*, starvation, amino acids

EXPLOITING POTENTIALS OF BACTERIAL ECOSYSTEMS IN CHEESE

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Cheese ripening is a complex process that is largely influenced by the microorganisms present in the cheese matrix. The diversity of cheese microbiota contributes greatly to the complexity of the cheese ripening process, which is of crucial importance for development of the unique organoleptic characteristics of each traditional cheese variety. Interactions between microorganisms, such as microbial competition, microbial cooperation and antagonistic effects, are of prime importance for the survival, growth and activity of microorganisms in cheese. Bacteriocins are antimicrobials produced by lactic acid bacteria that enable them to dominate the cheese ecosystem. Their potential as biopreservatives in cheese is well known. On the other hand, much less attention has been paid to the possible use of some bacteriocins to promote lysis of other lactic acid bacteria and hence contribute to cheese proteolysis and flavour development.

This presentation will review some of the strategies proposed to achieve control of the microbiota present in the cheese ecosystem as a means of ensuring high quality and safety standards in the production of cheeses. In particular, strategies such as selection of defined starter/adjunct systems that include strains with interesting, enhanced or complementary enzymatic activities, and the use of bacteriocins to modulate the rate of bacterial lysis during cheese ripening will be discussed.

Keywords: microorganism, ecosystem, cheese ripening

FERMENTATION OF CARBOHYDRATES FROM CHEESE SOURCES BY NON-STARTER LACTIC ACID BACTERIA ISOLATED FROM SEMI-HARD DANISH CHEESE

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Carbohydrate fermentation by non-starter lactic acid bacteria (NSLAB) isolated from Danish semi-hard cheese was studied using BioScreen C equipment. Of 45 isolates studied, 39 were identified as *Lb. paracasei/casei*, 2 as *Lb. curvatus* and 4 as *Lb. danicus* using ITS-PCR. Carbohydrate fermentation patterns were analysed by API-50CH. A specially designed carbohydrate restricted media (CRM) added individual carbohydrates (lactose, glucose, galactose, mannose, ribose, N-acetylglucosamine, N-acetylgalactosamine, N-acetylneuraminic acid and N-acetylmuramic acid) was used. Carbohydrates were selected as potential sources that could be found in cheese (free sugars, sugars from milk-fat globule membrane (MFGM), glycomacropeptide (GMP) or lysed cells).

Results showed that *Lb. paracasei* were able to grow on the main milk carbohydrates from MFGM and GMP as well as from peptidoglycan of bacterial cell walls but ribose was not a preferred carbohydrate. The highest growth rate was observed on N-acetylglucosamine (0.32–0.56 h^{-1}) and lowest on ribose (0.12–0.23 h^{-1}) if fermented. Nearly half of the ribose-positive strains according to API-50CH were not able to use ribose in BioScreen tests. These strains grew also slowly on CRM-ribose agar plates (pH 5.8, 2.5 % NaCl) incubated anaerobically at 12 °C. Half of the *Lb. paracasei/casei* strains were able to use N-acetylgalactosamine and 30 % N-acetylmuramic acid. None of the tested strains fermented N-acetylneuraminic acid.

On the contrary, *Lb. danicus* indicated better growth on carbohydrates from dead cells than from most of the milk carbohydrates of MFGM or GMP in cheese. *Lb. danicus* strains grew at 30 °C only in ribose CRM, but at a lower temperature (24 °C) also in CRM with N-acetylglucosamine, glucose, lactose or galactose and the fastest growth was observed on ribose (0.23–0.27 h^{-1}).

Keywords: non-starter lactic acid bacteria, cheese carbohydrate fermentation

ANTIMICROBIAL ACTIVITY OF GENETICALLY AND PHYSIOLOGICALLY CHARACTERIZED *LACTOBACILLUS* ISOLATED FROM SEMI-HARD CHEESES

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Non-starter *Lactobacillus* dominate the microbial flora in semi-hard cheese during the main part of ripening and they may affect cheese quality by competing with detrimental bacteria using nutrients or producing antimicrobial substances. In this study, about 1000 isolates from Danish semi-hard cheeses of different varieties, age and origin were analysed by ITS-PCR profiles. Approximately half of the isolates were shown to be *Lactobacillus*. The *Lactobacillus* isolates were classified using Pulse Field Gel Electrophoresis (PFGE), resulting in 135 different PFGE profiles or *Lactobacillus* strains. Sugar fermentation patterns for *Lactobacillus* isolates with different PFGE profiles were tested by API 50 CHL analysis. Screening for anticlostridial activity of the *Lactobacillus* strains against 15 isolates of *Clostridium* from milk, cheese and silage was performed by using agar well diffusion assay. Lytic activities of the *Lactobacillus* strains were tested against autoclaved *Lactococcus* in rich MRS-medium and in carbohydrate-restricted medium.

Of 147 *Lactobacillus* strains screened for their ability to inhibit *Clostridium*, one third had antimicrobial effect. Nine strains had a broad anticlostridial effect inhibiting 5-11 *Clostridium*. 17% of the *Lactobacillus* strains had the ability to lyse autoclaved *Lactococcus* cells, most of them only in carbohydrate-restricted medium. These strains were typically not the same as those that had anticlostridial activities demonstrating flexibility in competition strategy. The results indicate that anticlostridial non-starter *Lactobacillus* could be useful as protective cultures against growth of *Clostridium* during ripening of semi-hard cheeses.

Keywords: Cheese, *Lactobacillus*, Antimicrobial activity, *Clostridium*, Lytic activity

DIVERSITY OF DOMINANT MICROFLORA IN COMTÉ CHEESE

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Comté cheese is a variety of hard cooked cheese with an „Appellation d'Origine Protégée" (PDO), manufactured from raw milk and complex starter cultures. Ripened cheeses exhibit different sensorial characteristics according to the dairies and the period at which they are manufactured. The mechanisms generating such a large variety remain largely to be elucidated. Microflora plays a major role in the elaboration of the final characteristics, but how does its diversity look like among cheeses and generate the diversity of final characteristics?

Lactic and propionic acid bacteria of the inner part of the cheese represent the most active microflora in Comté cheeses with respect to the elaboration of their final characteristics. About 1600 isolates of lactic and propionic acid bacteria were collected at nine stages of manufacture and ripening (including raw milk and starter cultures) from two Comté cheeses exhibiting different sensorial properties. They were identified at species and strain levels by PCR techniques. In each cheese and for each species several strains were isolated, with one or two largely dominant. Although the same genus and species were identified in each cheese – 6 and 11 different respectively –, patterns and dynamics of dominant strains were cheese specific. The microflora was thus complex in each cheese in term of number of different species and of different dynamics.

The results confirmed previous reports on lactic acid bacteria in Comté cheeses. The strains originated either from milks or starter cultures, which can only partially explain their dynamics. But interactions between strains in environmental conditions that vary as ripening progressed should be considered in the future.

Keywords: Comté cheese, lactic acid bacteria, propionic acid bacteria, diversity

CHARACTERIZATION OF THE MICROFLORA IN SCANDINAVIAN CHEESE DURING RIPENING

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One of the most popular cheeses in Norway is the semi hard cheese named Norvegia and in Sweden the semi hard cheese named Präst. Norvegia and Präst are both cheese varieties made with a mesophilic DL-starter including strains of *Lactococcus* and *Leuconostoc.* The commercial manufacture of Norvegia and Präst are controlled processes that utilizes pasteurized milk in dedicated plants under hygienic conditions. However, despite the application of different precautions during cheese-making a secondary non-starter bacterial population develops in the curd. Non-starter lactic acid bacteria (NSLAB) is a collective term used to describe the adventitious LAB flora capable of growth under the selective conditions of ripening cheese. The objective of this study was to evaluate the diversity of the microflora in Norvegia and Präst cheese according to different dairies and different ripening stages.

Microbiological sampling of Norvegia and Präst cheese from three Norwegian and three Swedish manufactures, respectively, was done during ripening. The evolution of total bacterial counts, lactococci, lactobacilli, enterococci, presumptive leuconostoc and pediococci was investigated after 30, 90, 180 and 270 days of ripening. Isolates (275) of non-starter lactic acid bacteria (NSLAB) from 9 Norvegia and 12 Präst cheeses after 90, 180 and 270 days of ripening were examined. The isolates were tested by physiological and biochemical assays, species-specific PCR and 16S rDNA sequencing.

In Norvegia cheese, *Leuconostoc* spp., most probably from the starter, and the NSLAB specie *Lactobacillus paracasei* dominated among the isolates after 90 days of ripening , however, after longer ripening *Lb. paracasei* dominated. In Präst cheese, the predominant NSLAB specie was *Lb. paracasei*. The development and evolution of the microflora in Norvegia and Präst varied according to dairy and ripening time.

Keywords: non-starter lactic acid bacteria (NSLAB), cheese, ripening, identification

STARTER LYSIS IN CHEESE: AN ESSENTIAL BUT COMPLEX PHENOMENON

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Bacterial autolysis can be defined as the breakdown of a cell due to the hydrolysis of the peptidoglycan network. Endogenous cell wall associated enzymes, autolysins, or prophage encoded enzymes, endolysins, can induce this «suicide» phenomenon. Bacterial lysis has been observed in many species, but the mechanisms are, in most cases, not fully elucidated. Following conclusive experimental data obtained in the last decade, dairy starters lysis is now regarded as an essential pre-requisite in cheese maturation, since intracellular starter enzymes, in particular peptidases, can then play their major role.

In this review, we will present:

i) Current knowledge regarding starter lysis in cheese and, in particular, the different ways used to detect and follow lysis *in situ*

ii) Current knowledge regarding the mechanisms involved, focussing mainly, but not exclusively, on the model most investigated: *Lactococcus lactis.* Recent advances concerning the molecular characterization of peptidoglycan hydrolases will be summarized (sequence, structure, regulation) as well as current knowledge about the relationship between lysogeny and lysis.

iii) The impact of lysis on the ripening indices of several cheeses and the different ways to control or induce increased lysis *in situ.* Briefly, the increase of free amino acids due to early lysis has been well documented in the literature, as well as the reduction of bitterness by hydrolysis of large hydrophobic peptides. However, the impact of lysis on the generation of flavour compounds by the subsequent catabolism of these amino acids needs to be clarified.

In conclusion, lysis of dairy starters has been well documented over the last few years, but many questions remain to be answered: origin of strain dependancy? natural induction of lysis in cheese? occurrence in cheeses other than pressed cheeses (what about lysis of surface flora in soft cheeses? in blue veined cheeses?…), the direct relationship between lysis and amino acid catabolism or lipolysis (very few reports)? and the outcome of intracellular compounds released through lysis like other enzymes, minerals, DNA constituants, pyruvate, co-factors… Do they have any role in the growth of other species or in cheese ripening reactions?

Keywords: starter lysis, cheese

PROTEOMICS USED TO ANALYSE ENZYMES IN CHEESE

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Bacterial ecosystems confer to cheeses organoleptic characteristics, during ripening, not only through the living cells (acidification of milk, production of organic acid compounds), but also through the action of proteins released into cheese as the result of cell lysis, especially different groups of enzymes. A relevant control of cheese quality would require a deeper understanding of the cell machinery responsible for the enzymatic reactions as they take place *in situ*.

The use of proteomic tools allows a global and dynamic view of the proteins expressed by the bacteria, i.e. their proteome, under various conditions of culture. Since an increasing number of bacterial genomes, notably those from lactic acid bacteria (lactococci, lactobacilli) and propionibacteria have been sequenced, and great progresses in protein sequencing have been performed, it is now possible to use proteomics to screen the enzymes in complex food systems, such as cheese.

For this purpose, the release of enzymes into Emmental cheese aqueous phase was investigated using a methodology based on: size exclusion chromatography, 2D electrophoresis and MALDI-TOF mass spectrometry and/or *de novo* sequencing by nanoscale LC-ESI-MS/MS tandem mass spectrometry. This methodology allowed us to identify five functional groups of proteins involved in: 1) proteolysis, 2) glycolysis, 3) stress response, 4) DNA and RNA repair and 5) oxido-reduction. These results revealed stress responses triggered by thermophilic lactic acid bacteria and Propionibacterium strains at the end of Emmental ripening.

Such a study offers other potential applications. The bacterial enzymatic battery could be screened *in situ* in various types of cheese or dairy products, to study the enzyme release and even enzyme activity throughout technological processes. In turn, metabolic pathways involved in organoleptic characteristic elaboration or in technological stress resistance could be orientated.

Keywords: cheese, proteomic, stress, proteolytic and glycolytic enzymes

LACTIC ACID BACTERIA PEPTIDASE ACTIVITIES: MODEL SYSTEM TO INVESTIGATE THE ROLE IN GRANA CHEESE RIPENING

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Cheese ripening is essentially characterised by enzyme activity accounting for the transformation of the curd's principal components into more simple substances. Firstly as a well defined cellular entity and secondly as enzymes released after cell lysis, lactic acid bacteria (LAB) are the main cause of this process of change. The intervention of a complex bacterial enzyme-based proteolytic system was supposed to ensure a progressive degradation of the casein-derived peptides into free amino acids and other compounds participating to flavour formation during the time. At present time this biochemical event is only deduced by the evidence of simultaneous presence and evolutions in cheese of shortened casein peptides and LAB peptidase activities in a stable form. As enzymatic activities could be induced or inhibited by different technological parameters of processing or ripening conditions, the presence of enzymatic activities was not enough to demonstrate the activity in cheese paste.

The aim of this work was to investigate in which phases of ripening of Grana cheese, LAB peptidase activities present in cheese extract could be involved in casein degradation. The ability to efficiently hydrolize N-terminal proteins residues was investigated varying pH of substrates, incubation temperatures and salt concentration to elaborate models useful to understand when these activities play a specific role in subsequently moments of cheese ripening.

Results evidenced that the different LAB peptidases are strongly conditioned by cheese processing and ripening parameters. Enzymes act at different time during ripening. The different condition present in the different cheese paste zones during the first period of ripening also conditioned enzymatic activity.

Keywords: peptidase activities, LAB, Grana cheese, model system

ROLE OF STARTER LYSIS IN CHEESE PRODUCTION

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Lysis of lactic acid bacteria plays a crucial role in dairy fermentations. The bacterial lysis ensures the involvement of the intracellular starter enzymes in the gradual process of cheese flavour formation. At NIZO food research we are investigating the lysis process in the following way: i) The comparison of highly stable and unstable strains in cheese manufacture to establish the role of starter lysis in the various stages of cheese ripening, and: ii) The role of growth conditions on the starter cell permeability and on the efficiency of starter lysis and its implication for cheese manufacture.

200 *Lactococcus lactis* strains were compared in cell stability and sensitivity of the cell walls for the lytic enzyme mutanolysin. In general, rapidly lysing strains showed high sensitivity of the cell walls for mutanolysin, while highly stable strains were more resistant. In cheese experiments, the rapidly lysing strains generated much more flavour components than the highly stable strains. Special attention was given to the accumulation and removal of the bitter-flavour in cheese. A direct correlation was seen between stability of cell(s)(walls) and the accumulation of bitter flavour in cheese. A range of specific debittering, flavour enhancing cultures, e.g. *L. lactis* NIZO B33 and AB28 with a high lysis efficiency, have been isolated and developed into adjunct starter cultures.

The effect of growth conditions on lysis efficiency was studied in a continuous culture. A highly labile strain (*L. lactis* AB28) and a highly stable strain (*L. lactis* AB8) were both cultivated at different growth conditions. In the labile strain, the cells, and the cell wall composition, became increasingly stable at lower growth rates, while the stable strain was not visibly effected by these changes. In addition, effects of growth temperature and pH on cell permeability were observed for both strains. Most of the observed changes in cell stability and/or permeability could be correlated with changes in the cell wall composition, in particular the peptidoglycan structure. These results indicate that the lysis process can indeed be controlled by choosing suitable growth conditions.

Keywords: lysis, cheese ripening, starter cultures

MECHANISMS OF INCORPORATION AND RELEASE OF ENZYMES INTO CHEESE DURING RIPENING

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This article will review current knowledge regarding the means by which enzymes are incorporated and released into cheese during ripening. Cheese ripening is catalysed by enzymes from: milk, coagulant, starter Lactic Acid Bacteria (LAB) and non-starter Lactic Acid Bacteria (NSLAB). Attempts to accelerate flavour development have also lead to the addition to cheese of exogenous commercial enzyme preparations including adjunct and attenuated LAB and NSLAB strains. The possible entry points for enzymes into cheese are via: milk, wash water, dry salt or brine. However, exogenous enzymes are generally added to cheesemilk or with dry salt to the curd. The contribution of enzymes present in cheese during ripening depends on: (1) the degree to which they entrapped in the curd (2) their distribution within the curd and (3) their activity under the physico-chemical conditions existing in the particular variety. Most indigenous milk enzymes are associated with the caseins or milkfat and partition with curd during cheesemaking providing well distributed residual enzyme activity. Little information exists on the mechanism of entrapment of LAB or NSLAB within the curd but it is believed they become physically entrapped during cheesemaking and localise at the interface between milkfat gobules and the casein matrix. Release of intracellular enzymes from starter LAB and NSLAB occurs through cell permeabilization or autolysis in a strain-dependent manner. In the case of exogenous enzyme preparations, the choice of entry point greatly affects both the extent of enzyme incorporation and distribution in the curd. For exogenous enzymes added to milk, considerable losses are experienced at whey drainage, hence to improve the extent of incorporation, various enzyme encapsulation techniques have been developed and these are also reviewed.

Keywords: enzymes, incorporation, release, encapsulation.

ACCELERATION OF PROTEOLYSIS IN CHEESE USING STREPTOKINASE, A PLASMINOGEN ACTIVATOR

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In milk, there are higher levels of plasminogen, the inactive precursor of plasmin than of the active proteinase. Streptokinase is an extracellular protein produced by the mastitis pathogen *Streptococcus uberis* which can activate plasminogen to plasmin. The aim of this study was to activate plasminogen to plasmin using a semi-purified preparation of streptokinase, or as starter a strain of *Lactococcus* (PRA270) which had been genetically modified to produce streptokinase, and to study effects of increased plasmin activity on proteolysis during cheese ripening. Cheddar cheese was manufactured from milk supplemented with a semi-purified preparation of streptokinase at 0.1%, 0.25 % or 0.50 % (v/v) and, in separate trials, miniature Cheddar-type cheeses were made using strain PRA270 as starter. Both approaches resulted in activation of plasminogen, with most occurring on the day of manufacture, hence increasing plasmin activity in experimental cheeses ~ 3 fold compared to control cheeses. Increased plasmin activity in the experimental cheeses resulted in accelerated proteolysis as indicated by higher levels of pH 4.6-soluble N, accelerated hydrolysis of β-casein and concomitant increases in concentrations of γ-caseins. Reversed-phase HPLC showed increased production of hydrophobic peptides in cheeses with higher plasmin activity; however, levels of free amino acids were unaffected. In conclusion, use of streptokinase, either as an exogenous semi-purified preparation or produced by the starter culture, activated bovine plasminogen to plasmin, which accelerated proteolysis in Cheddar cheese during ripening.

Keywords: Plasmin, streptokinase, genetically-modified starter, proteolysis, cheese

INJECTION OF STARTER BACTERIA AND RIPENING ENZYMES INTO A CHEESE MATRIX

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Adding starter bacteria and rennet to milk makes cheese, and during maturation the cheese characteristics develops. However, adding starter bacteria, adjunct ripening bacteria or enzymes directly to the cheese matrix improves ripening control and may even accelerate ripening (patent WO0180655, 2001). In addition this method reduces the loss of ripening agents in the whey and for that reason enhances the quality of the whey.

We made semi-hard cheeses from milk acidified to pH 6.4 with lactic acid. Just before final pressing we used a needle device to inject a suspension of starter (*Lactococcus lactis*) into the cheese matrix. Adjunct bacteria (*Lactococcus* and *Lactobacillus species*) and enzyme (serine protease), respectively was injected to accelerate ripening. We evaluated ripening by pH 4.6 soluble N analysis, casein degradation by Capillary Electrophoresis (CE), and peptide profiles by reversed phase High Performance Liquid Chromatography (rp-HPLC). For comparison we analysed a commercial nine-month-old Cheddar cheese.

Cheese pH decreased to 5.1–5.3, and no marks were detected from the needle penetration. Cheese containing starter bacteria and adjunct bacteria approached similar level of pH 4.6 soluble N (22.1 %) after 3 weeks as in a 9 months old Cheddar cheese (21.2 %). However, cheeses with enzyme increased soluble N to 35–45 % of total N. CE revealed, that α_{S1}-and β-caseins were totally degraded in the latter cheeses, while more than 50 % of casein was present in the cheeses without ripening enzyme. Total peak area in the HPLC chromatograms reflected the comprehensive casein degradation, as enzyme treated cheese showed the highest area. However, hydrophilic peptides accounted relatively more to total peptide profile in cheese with adjunct bacteria, indicating release of peptidases to the cheese matrix.

Full-scale cheese trials with combination of ripening enzyme and adjuncts are in progress.

Keywords: Technology, Adjunct, Incorporation, Peptides

OVERPRODUCTION OF CYSTATHIONINE β-LYASE IN *LACTOCOCCUS LACTIS* AFFECTS FLAVOUR DEVELOPMENT IN GOUDA CHEESE

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An attractive approach to accelerate flavour development in Gouda cheese is to overproduce in *L. lactis* cystathionine β-lyase (CBL), an enzyme involved in conversion of methionine into volatile sulphur compounds present in several cheese types. A comparative *in vitro* expression study showed that the CBL enzyme harboured the highest activity towards methionine in comparison with other predicted lactococcal C-S lyases. Fifty-fold overproduction of specific CBL-activity was achieved by controlled expression of the *metC* gene, encoding CBL, of *L. lactis* B78 using the food-grade nisin-inducible expression system (NICE™). Cheese graders awarded an additional sulphur/thermophilic flavour to Gouda cheese prepared with the CBL-overproducing strain as an adjunct. In an attempt to accelerate flavour development we manufactured Gouda cheese which, together with the CBL-overproducer, also contained a *L. lactis* strain producing the lysin and holin proteins of lactococcal bacteriophage US3. Production of lysin and holin leads to *in trans* lysis of surrounding bacteria during cheese ripening, thereby increasing the amount of released CBL in the cheese matrix two-fold. However, sensory analysis showed no significant effect of enhanced cell lysis on cheese flavour development. A possible explanation for this result is that part of CBL present in cheese extracts is found to be inactive, probably due to dilution of its cofactor pyridoxal-5'-phosphate into the cheese matrix upon cell lysis.

Keywords: cystathionine β-lyase, food-grade overproduction, cheese trials, sulfur flavour

APPROACHES TO MAXIMISING RECOVERY OF MILK CONSTITUENTS DURING CHEESE MANUFACTURE

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This review will consider milk compositional and processing factors influencing the efficiency of recovery of milk constituents in the manufacture of rennet curd cheeses. Compositional factors will include genetic polymorphs of milk proteins and other factors influencing curd formation which impact on the retention of fat and protein during cheese manufacture. Processing variables influencing cheese yield in routine manufacture will be considered. Novel technologies such as high pressure, microfiltration and ultrafiltration and their potential use in maximising protein or fat recovery in curd will be considered together with the effects of these processes on the syneretic properties of curds and the ripening characteristics of the resultant cheese.

Keywords: Cheese yield, manufacture, genetic polymorphs

USE OF COLD ULTRAFILTERED RETENTATES FOR STANDARDIZATION OF MILKS FOR PIZZA CHEESE: IMPACT ON YIELD AND FUNCTIONALITY

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A washed, stirred-curd cheese was manufactured from two types of UF fortified milks: high-solids (HS; 15.4% TS, 4.5% casein), and medium-solids (MS; 13.5% TS, 3.6% casein). Cheesemilks (casein:fat ratio ~ 1.0) were obtained by blending cold processed UF retentate (27.5% TS, 8.3% casein, 12.2% fat) with partially-skimmed milk and UF (skim milk) retentate (11.3% TS, 3.9% casein, 0.2% fat). Control cheese was made with partially-skimmed milk (11.3% TS, 2.5% casein). Coagulation at 34 °C was monitored by dynamic low-amplitude oscillatory rheology (DLAOR). Cheese functionality was assessed using DLAOR and when baked on a pizza. Gels made from UF-fortified milks had similar clotting times and were faster (16–17 vs 22 min) than control milk. Shear stress values of gels were 62, 50 and 25 Pa for HS, MS and control milks, respectively. MS cheese had lower moisture contents (45.6%) than control (47.6%) or HS cheeses (47.7%). Fat recoveries in cheeses were higher in MS cheeses (92.51 ± 0.86) than control cheeses (90.26 ± 1.35) but lower in HS cheeses (88.57 ± 0.83). Nitrogen recoveries were lower in control cheeses than in UF-fortified cheeses. Loss tangent curves (at temperatures > 40 °C) shifted higher as cheese aged up to a month and decreased with further ripening. Temperature for the loss tangent decreased with age up to 2 mo for all three cheeses types, thereafter it did not change much. Loss tangent values were slightly lower in UF-fortified cheeses. TCA-soluble nitrogen levels were similar in all three cheeses. When the cheeses were baked on pizzas, only slight differences were noted between the cheeses. In conclusion, standardization of milk with cold UF retentates can be used to increase cheesemaking productivity without adversely affecting cheese functionality.

Keywords: UF, non- pasta-filata pizza cheese, cheese yield, cheese functionality

CARBOHYDRATE-BASED FAT REPLACERS AND FUNCTIONALITY OF LOW FAT MOZZARELLA CHEESE

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Reducing the fat content in Mozzarella cheese results in a lower ratio of moisture to protein leading to reduction in functionality of cheeses. Reduction in fat content also results in an inferior quality cheese, specifically poor melt and stretch and the cheese becomes harder with rubbery texture. Carbohydrate-based fat replacers has been used to increasing the moisture content and consequently improving the functional properties of the cheese. Skim milk Mozzarella cheeses (<3% fat) were made using two maltodextrin based (Maltrin M1 and M2) and a modified potato starch based (StaSlim S1) fat replacers. A control batch was made with skim milk without any fat replacers. The texture characteristics of the cheeses were measured with an Instron Universal Testing Machine and the microstructure examined using scanning electron microscopy. The moisture contents of the skim milk and Maltrin-based cheeses were similar, while StaSlim S1 based cheeses showed lower moisture levels. The protein contents of the cheeses made with fat replacers were lower than the control cheeses. The Maltrin based cheeses were lower in hardness than the control or StaSlim based cheeses and the hardness decreased during storage, while the StaSlim-based cheeses became harder during storage. In general, the cheeses made using fat replacers showed less cohesiveness and springiness than the control cheese, while adhesiveness increased. The Maltrin-based cheeses showed significantly less gumminess and chewiness, however, these values for StaSlim-based cheeses increased during storage. Incorporation of fat replacers resulted in an increased openness in cheeses, and large serum channels (up to 0.1mm diameter) were seen. The Maltrin-based cheeses showed more openness than the StaSlim-based cheeses. However, carbohydrate-based fat replacers do not behave like fat, particularly when heated. The pizza bake performance for such cheeses showed poor results in regards to melting and fusion of shredded cheese used as pizza topping. High degree of surface scorching was also observed.

Keywords: Low fat, Mozzarella cheese, fat replacer

ECONOMIC ASPECTS OF CHEESE MAKING, INCLUDING COMPARISONS TO HIGH VALUE WHEY PRODUCTS

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Cheese making inevitably entails the production of whey. The economics of processing this liquid fraction is for a great deal dependant on capital investments which are much more dependant on scale of economics, than cheese making itself. Small cheese plants with daily milk throughput of approximately less than 100 000 kg cannot economically justify the capital for water removal equipment. For small plants which have to convert the whey to dry product, this can be done by pre-concentrating with a reverse osmosis unit or a small plate evaporator and drying on a double roller dryer. the economics are evaluated at several price levels. At the upper scale of cheese plant size at 2 to 3 million kg/d of milk, the investment for whey processing is about half the total investment. Returns on investment are calculated for several, electricity and natural gas prices and at varying whey powder prices. Increased investment for further processing into whey protein concentrate and dried whey solubles or lactose is evaluated at several price levels.

Keywords: Economic aspects, cheese making, whey products

ABSTRACTS
OF POSTER PRESENTATIONS

P001 PARTICULAR TREATMENTS TO ACHIEVE TYPICAL FLAVOURS IN TWO PECULIAR SEMI-HARD ITALIAN CHEESES

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In the North East of Italy small amounts of few characteristic cheeses are produced with typical flavours that can be achieved only by particular treatments. For instance, the Asino cheese is a traditional dairy product of some areas of Friuli Venezia Giulia, obtained by normal cheeses (soft or semi-hard) that, after a normal salting, are dipping in a special diluite salting brine, mixed with whey, milk and milk cream, called „salmuerie". So Asino cheeses acquire their own typical flavour. This research contribute to the description of the biochemical processes thank to which the Asino cheese has the peculiar sensorial characteristics that make this cheese different from similar cheeses ripened in warehouses without dipping in „salmuerie". Particularly, in all the „salmuerie" analyzed there was a fair presence of short chain free volatile fatty acid that remains almost steady for all the time in which the cheese is dipped into. Instead the free volatile fatty acids in cheese starts from values very low and then rises and reaches a balance with the salmuerie values. An other interesting product that we are considered in this research was a cheese dipping, after ripening, in wine or must and marc. We have showed that components of wine migrate to cheese by an exchange process that gives to this product typical taste and smell. Actually, after dipping in wine was found fairly amounts of ethyl acetate component, that give to cheese characteristics floral flavours.

Keywords: semi-hard cheeses, salting, biochemical processes, flavour

P002 THE EFFECT OF MOISTURE AND RIPENING TIME ON MODEL CHEESE TEXTURAL PROPERTIES AND PROTEOLYSIS

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Uniaxial compression was used to measure the effect of moisture and ripening time on model cheese texture. This allowed a universal approach to cheese characterisation, because properties that are well defined in rheology and method protocols used in the international community were utilised.

Knowledge of the textural changes induced by moisture can assist the development of new cheeses of different moisture contents and defined textural attributes. Two sets of model cheeses with moisture ranges of 34–39 % (pH 5.51–5.54) and 40–48 % (pH 5.76–5.80) were made using the acidulant glucono-δ-lactone to minimise pH changes within a set of model cheeses.

The rheological properties predicted by moisture (averaged over 2–184 days of ripening time) changed as follows. As the moisture increased from 40 to 48 %, fracture strain (longness) increased 2.1 fold, fracture stress (firmness) decreased to 54 %, modulus (stiffness) decreased to 30 % and adhesion area (adhesiveness) increased 44 fold, compared with the value at 40 % moisture. Similarly, from 34 to 39 % moisture, fracture strain increased 1.9 fold, fracture stress decreased to 81 %, modulus decreased to 73 % (with slightly non-linear trends) and adhesion area gave non-linear trends. The cheddaring temperature (which induced moisture changes) had an effect ($P < 0.05$) that depended on the ripening time for fracture strain, modulus and adhesion area (at 40–48 % moisture) and for fracture strain and adhesion area (at 34–39 % moisture).

α_{s1}-Casein breakdown was faster at higher moisture, probably because chymosin was more active (and cheddaring temperature had an effect [$P < 0.0005$] that depended on the ripening time for 40–48 % moisture cheeses). β-Casein breakdown was either faster for higher moisture in the 34–39 % moisture cheeses or similar at all moistures in the 40–48 % moisture cheeses. Moisture had no significant influence on non-protein nitrogen per total nitrogen levels (cheddaring temperature had no effect [$P > 0.05$]).

Keywords: cheese moisture, texture, rheology, ripening time

P003 AUTHENTICATION OF PDO OVINE CHEESES. IDENTIFICATION OF THE USE OF VEGETABLE COAGULANT (*CYNARA* L)

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Some of the traditional ovine cheeses produced in the Iberian Peninsula use the aqueous extracts from dried cardoon flowers (*Cynara* L.) as coagulant. Some of them carry the label of Protected Designation of Origin (PDO), making compulsory the use of local pure ovine raw

milk and cardoon (usually *C. cardunculus* L.) as coagulant. However, their increasing consumer demand might lead to the fraudulent addition of milk from other species to extend production and/or the substitution of the vegetable by other conventional coagulant. Detection of milk mixtures in cheese has been done by different methods, but detection of the coagulant origin in cheese is not yet possible. A Capillary Zone Electrophoresis (CZE) method has been applied to a cheese made with a vegetable coagulant, which was compared to other cheeses made with animal rennet and microbial coagulant. The results obtained led us to suggest that CZE of cheese caseins is a suitable, easy to perform and fast method for evaluating the authenticity of cheeses made with *Cynara* L. A peak, probably arising from β–casein, has only been detected in cheeses coagulated by cardoon and it is not proteolysed during maturation. This peak could be the distinguishing factor of the type of coagulant employed.

Keywords: Authentication, PDO Ovine Cheese, Identification, Vegetable Coagulant, *Cynara* L

Part of this work was presented as a poster at Congrilait, held in Paris from 24th to 27th September 2002. Complete work was submitted to Le Lait in October 2002

P004 FLAVONOID GLYCOSIDES FROM THE VEGETABLE COAGULANT *CYNARA* L. AS MARKERS FOR PDO CHEESES (PRELIMINARY RESULTS)

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The majority of the PDO ovine cheeses produced in Portugal use the aqueous extracts from dried cardoon flowers (*Cynara cardunculus* L.) as coagulant, which is prepared by the cheesemaker just before use. This purple-brownish colour aqueous extract is completely added to the milk, and apart from its proteolytic enzymes (cardosins) nothing is known about other components present therein.

Aqueous extracts from air-dried flowers of cardoon were analysed by HPLC-DAD for the presence of flavonoids, and the results obtained revealed the presence of two major peaks characterised as apigenin-7-*O*-glycosides, one of them being identified as isorhoifolin (apigenin-7-*O*-rutinoside).

Model cheeses made from raw cow milk and cardoon, raw cow milk and animal rennet and raw cow milk and animal rennet plus isorhoifolin as standard were made, in order to detect the flavonoids characteristic from the vegetable coagulant in cheese.

HPLC-DAD analysis of curds and respective wheys revealed that the curd obtained with cardoon presented the same two major peaks detected in the coagulant. isorhoifoline was also present in the curd obtained with animal rennet plus added standard, but none of the flavonoids found in the vegetable coagulant were present in the curd obtained with animal rennet. No evidence of these flavonoids in any of the wheys revealed that they are almost completely retained in the curd.

These preliminary results lead us to suggest that the two apigenin-7-*O*-glycosides characteristic from the vegetable coagulant, specially isorhoifolin, could be used as markers for the authentication of PDO cheeses made with cardoon.

Keywords: cheese authentication, vegetable coagulant, isorhoifolin, authenticity markers

P005 RIPENING PROFILE OF URFA CHEESE (A TURKISH WHITE-BRINED CHEESE) PRODUCED BY TRADITIONAL WAY AND BY ULTRAFILTRATION TECHNIQUE

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The development of ripening in white-brined Turkish cheese (Urfa type) produced by traditional way and by ultrafiltration (UF) technique was studied. The effects of scalding on the development of proteolysis and texture of cheeses were also investigated. Variations in the nitrogeneous fractions and textural characteristics were investigated throughout 90-day storage at 4 °C.

Results obtained showed that the proteolysis developed faster in the UF-cheese than the cheese manufactured by traditional way. The UF sample had higher levels of water soluble nitrogen, proteose-pepton nitrogen, non-protein nitrogen and ripening coefficients than the traditional cheeses. Scalding slowed down the development of proteolysis in both group of cheeses, however, the trend of ripening remained almost unchanged. SDS-P.A.G.E. electrophoretograms showed that the breakdown of α_s- and β-casein was more remarkable in the unscalded UF cheese. SEM pictures revealed that the UF sample had denser microstructure than the traditional cheese and scalding led to even much denser structure. In accordance with microstructure of samples, the textural properties of UF cheeses were higher than that of traditional cheeses. The hardness and springiness of the samples increased rapidly during the first 15–20 days of storage and then

continued to increase with a decreasing velocity during the rest of the storage. The UF cheeses (both scalded and unscalded) had springer bodies than their traditional counterparts.

To conclude, it can be said that by employing UF technique, it may be possible to omit dry salting prior to brining and scalding steps from the manufacturing practices of white-brined Turkish cheese (Urfa type).

Keywords: Turkish white cheese, proteolysis, texture

P006 CHIHUAHUA CHEESE: MICROBIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES

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Chihuahua Cheese is a semi-hard cheese that is highly desired and it is consumed fresh, usually within two months after manufacture. It is produced by Mennonite communities in the State of Chihuahua, Mexico, and is considered a unique style of cheese, though it is a variation of Cheddar cheese. Daily production is more than 55 000 kg in 23 small cheese factories. Only a small amount of literature has been published on the microbiological content and physicochemical properties. This investigation determined the sanitary quality and physicochemical characteristics of this Chihuahua cheese manufactured from raw milk. Samples of cheese were obtained from 9 cheese factories within a few days of being manufactured during the year 2002-03. The compositional properties of the fresh cheese were similar among all manufacturers. Composition averaged 40 % moisture, 21 % protein, 28 % fat, 1 % salt and the pH was 5.6. All cheeses tested negative for *salmonella spp*. Fecal coliforms counts ranged from 2 to 8 $\log_{10}$ cfu/g for raw milk cheese. Yeast and mold counts ranged from 3 to 5 $\log_{10}$ cfu/g and S*taphylococcus aureus* was found in the cheese studied. Establishing the basic chemical, microbiological and physical properties of Chihuahua cheese, is the first step in understanding its unique traits and exploring ways to improve quality and expand consumption.

Keywords: Chihuahua cheese, Mennonite, physicochemical properties

P007 CHARACTERIZATION OF COMPOSITIONAL AND MICROBIAL PROPERTIES OF CHIHUAHUA CHEESE MANUFACTURED FROM RAW AND PASTEURIZED MILK

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Chihuahua Cheese is a fresh and semi-hard cheese traditionally made in the State of Chihuahua, Mexico, and manufactured primarily by the Mennonite community. It is highly desirable, and is considered a unique style of cheese, though it is a variation of Cheddar cheese. Daily production in Chihuahua is more than 130 000 kg in small cheese factories. Little quantitative data has been published on these Cheese. This study characterizes and compares the physicochemical and microbial properties of Chihuahua cheese either from raw or pasteurized milk. Samples of cheese were obtained within a few days of being manufactured from nineteen cheese factories during the year 2002 and 2003 (14 raw and 5 pasteurized milk cheeses). The compositional properties were similar among cheese manufactured from raw and pasteurized milk. Overall composition averaged 41 and 40 % moisture, 21 and 20 % protein, 28 and 29 % fat, for cheeses made from raw and pasteurized milk, respectively. Color characteristics (L, a, b values) for cheeses from raw milk were 81, –2.70, and 16.70, respectively; and 85, –2.40 and 23.70 for pasteurized milk cheeses. Fecal coliforms counts ranged from 2 to 6 $\log_{10}$ cfu/g for raw milk cheeses, and 0.30 to 6 $\log_{10}$ cfu/g for pasteurized milk cheeses. Yeast and molds counts ranged from 3 to 7 $\log_{10}$ cfu/g for raw milk cheeses, and 2 to 6 $\log_{10}$ cfu/g for pasteurized milk cheeses. *Staphylococcus aureus* was found in raw milk cheeses, and was not found in pasteurized milk cheeses. Both types of cheeses tested negative for S*almonella spp*. Further studies is needed to identify and explain which raw and pasteurized milk factors, and processing steps are key factors in producing quality traits of Chihuahua cheese. We urge to use pasteurized milk to avoid the possibility of human diseases due to pathogens in raw milk.

Keywords: Physicochemical characteristics, raw milk, pasteurized milk, Chihuahua cheese

P008 DETECTION OF LOW LEVELS OF COW MILK IN BUFFALO MOZZARELLA CHEESE BY MEANS OF IEF AND PCR METHODS

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Buffalo Mozzarella has to be made exclusively with pure buffalo milk and any milk ingredient from other species has to be absent. According to EEC regulation 213/2001, it is assumed that cows' milk is present if the apparent cows' milk casein content of the sample to be analysed is equal to or higher than 1 %. The principle of the EU reference method is based on the different mobility exhibited by isoelectric focusing gel analysis (IEF) of buffaloes' and cow's γ2 and γ3 caseins resulting from the activity of plasmin towards the caseins extracted from the cheese.

However, even if cheese-makers claim the exclusive use of buffalo milk, sometimes they have been charged with the presence of cow milk in Buffalo Mozzarella near to the detection limit of the method.

IEF analyses of pure buffalo milk and Mozzarella showed a band with mobility similar to the cow's γ2 casein. To assess the purity of these samples, a Polymerase Chain Reaction (PCR) technique, that amplifies *cytochrome b* mitochondrial gene, has been applied. PCR results confirmed the purity of buffalo milk. The sensitivity of the method, applied to detect the addition of cow's milk to buffaloes' milk, was higher than 0.05%. When applied to Buffalo Mozzarella cheese obtained from pure buffaloes' milk or added with 1, 2 or 3 % of cow milk, PCR technique confirmed its reliability, correctly discriminating pure from adulterated samples.

In conclusion, the presence in both, milk and Mozzarella cheese of a band with IEF mobility similar to the cow's γ2 casein, having a peak area near to that given by samples obtained by addition of 1 % cow milk, it is not necessarily a proof of buffalo milk adulteration. Studies are necessary to identify the nature and the origin of this band.

Keywords: species identification, IEF, PCR, buffalo

P009 DETECTION OF FOREIGN FATS IN COWS', EWES' AND GOATS' MILK CHEESES DURING RIPENING

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The triacylglycerides (TAG) composition of different cheeses with a Protected Designation of Origin (PDO) was studied for detection of foreign fat in milk fat. Mahon cheese (51 samples) made using pure cows' milk and Manchego cheese (51 samples) with pure ewes' milk were manufactured in either artisanal or industrial cheese factory and matured for 2, 4 and 6 months. Cabrales cheese (12 samples) was industrially made with pure cows' milk and artisanal Majorero cheese (12 samples) with goats' milk and ripened for 1, 2 and 3 months. TAG were separated by gas chromatography, using a short capillary column and analyzed according to their carbon number. Multiple regression equations based on TAG composition proposed to detect foreign fats in cows' milk fat (Precht, 1991; EC, 2001); in ewes' milk fat (Goudhil et al., 2003); and goats' milk fat (Fontecha et al., 1998) had been applied.

In Mahon and Manchego cheeses where the lipolysis was feeble, the TAG composition did not change substantially during ripening. The values obtained for the applied formula for cows' and ewes' milk respectively maintain its range. Therefore this equations can consider useful for detection of foreign fat in this cheeses during the studied ripening period.

Nevertheless, Cabrales (blue cheese) and Majorero cheese (made with pregastric rennet pastes), showed during the ripening period a extensive lipolysis and changes in the TAG profile. Therefore in these cheeses, the applied formula become inadequate due to that results would indicate the presence of foreign fat.

Keywords: foreign fat, cow, ewe, goat, triacylglycerides

P010 MICROSTRUCTURE OF CHEESE: AN OVERVIEW

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Cheese has been the focus of countless research efforts, which have contributed to the success of the cheese industry. On account of this, it becomes imperative to further understand its physico-chemical properties, in order to better describe the whole set of parameters that may influence its microstructure.

Knowledge of the microstructure of cheese may effectively indicate which factors to improve in industrial practice of cheesemaking. It is important to establish validated relationships between quality and safety of such product, in order to improve consumers' trust and perception. It is also important to tackle the possibility of innovation in terms of development of alternative products and processing thereof. Sensory analysis is also affected by structural alterations in the cheese matrix. Thus, it is essential to correlate all such parameters so that innovation may result from a deeper knowledge of microstructure. This discipline has been developed over the past four decades by several researchers, and several publications on this subject already are available. A set of important features on the microstructure of cheese will be reviewed in this communication.

Keywords: cheese microstructure, cheese matrix

P011 STUDY OF THE GEOGRAPHIC TRACEABILITY OF EUROPEAN EMMENTAL CHEESE TYPES USING FRONT FACE FLUORESCENCE SPECTROSCOPY

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Nowadays, objective and authentic food information is a major concern of many consumers, and it is gaining importance. Labelling and compositional regulations, which may differ from country to country, have a fundamental place in determining which scientific tests are appropriate for a particular issue. Cheeses with origin identification are generally high-priced and bring in a higher benefit to the producers than ordinary cheeses. For consumers having an extensive choice of food commodities, authenticity is a determinant criteria for guaranteering quality.

Spectroscopic techniques are fast, relatively low-cost and provide a great deal of information with only one test. They are considered as non-destructive, rapid, environmentally friendly and non-invasive, which makes these methods suitable for on-line or at-line process control. Front-face fluorescence spectroscopy coupled with chemometric techniques is very promising in this context. This technique was investigated for its potential for discriminating Emmental cheeses originating from various geographic origins. A total of 72 Emmental cheeses, produced during summer, from five countries, i.e., Finland (n=4), Germany (n=6), Austria (n=8), France (n=27) and Switzerland (n=27) were analysed. Protein tryptophan and vitamin A fluorescence spectra were recorded directly on cheese samples. Considering tryptophan fluorescence spectra, a good classification was observed for 76.4% and 63.5% of the calibration and the validation samples, respectively. A better classification was obtained from the vitamin A fluorescence spectra since 93.9 and 90.5% of the calibration and validation spectra, respectively were correctly classified. Cheeses from Finland were well separated using tryptophan or vitamin A fluorescence spectra. Considering cheeses from Germany, 100% of good classification was only obtained with vitamin A spectra. Cheeses from Austria, France and Switzerland were generally discriminated, but several samples were incorrectly classified. It was concluded that vitamin A fluorescence spectra may be considered a promising probe for the reliable evaluation of Emmental cheese origin.

Keywords: Emmental cheese, geographic origin, fluorescence, chemometric

P012 RUMENIC ACID AND ITS PRECURSORS IN INTERMEDIATE AND FINAL PRODUCTS OF THE CHEESE MAKING

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Coniugated linoleic acids (CLA) have been recognised to possess several biological activities. C18:2 9-cis, 11-trans (rumenic acid) the most representative CLA isomers, is abundant in dairy products from ruminant animals and in particular in sheep milk.

The aim of this work was to verify the influence of two different cheese-making technologies on the concentration of rumenic acid and its precursors in the fat of ewe's milk, cheese, ricotta cheese, whey, and *scotta*-whey. In each experiment, a batch of ewe's bulk milk was divided into two vats and processed following two different techniques to obtain a soft and a semi-hard cheese. The fatty acids of milk, final products and their intermediates were analysed by gas-chromatograpy.

Different technologies significantly affect only the final concentration of rumenic acid in the *scotta*-whey ($P < 0.05$). In semi-hard cheese process, the concentration of rumenic acid in fat was significant higher ($P < 0.01$) in whey (14.16 mg/g of fat) and *scotta*-whey (13.79 mg/g of fat) than milk (12.42 mg/g of fat), cheese (12.17 mg/g of fat) and ricotta cheese (12.52 mg/g of fat). Vaccenic acid showed a similar result ($P < 0.01$) with a concentration of 28.35 mg/g of fat in whey, 27.78 in *scotta*-whey, 24.61 in milk, 24.51 in cheese and

24.96 in ricotta cheese. The results also showed a tendency of the other rumenic acid precursors (C18:2 and C18:3) to accumulate in the cheese making intermediates whey and *scotta*-whey.

In conclusion the concentration of rumenic acid and its precursors in cheese and ricotta cheese is not affected by different technologies. In the semi-hard cheese making-process, this concentration increase in the cheese-making intermediates as the whey and the *scotta*-whey that could be used as raw materials to produce new dairy products naturally enriched in CLA.

Keywords: Rumenic acid, precursors, ewe's milk, cheese making

P013 MICROBIOLOGICAL AND CHEMICAL CHARACTERISATION OF A TYPICAL ITALIAN CHEESE: THE „ROBIOLA DI ROCCAVERANO" PDO

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The purpose of this study was the microbiological and chemical characterisation of Robiola di Roccaverano, a typical Italian cheese (Piedmont region, Val Bormida) with Protected Denomination of Origin (PDO).

A comparison between Robiola di Roccaverano produced by artisanal process using raw goat milk and by industrial process with mixed bovine-goat milk was made.

Seasonal (Summer and Winter 2002, Spring 2003) Robiola samples (fresh and ripened for 20 days) were collected from 1 industrial and 4 artisanal producers. For each sample several chemical parameters (gross composition, total and free fatty acids, electrophoretic pattern of caseins, some amino acids) were analysed. Total mesophilic and termophilic bacteria, LAB (Lactic Acid Bacteria), gram negative bacteria and fungi were enumerated and the presence of biogenic amines producer strains (histamine, phenylethylamine, tryptamine, tyramine) and of pathogenic bacteria (*Listeria, Salmonella, Staphylococcus aureus*) was investigated.

The statistical analysis (ANOVA) of the comparison between artisanal and industrial production showed a significant difference of LAB seasonal enumerations among the artisanal samples of Robiola. This result underlines the influence of the different processes and conditions of cheesemaking in the cheese microflora composition. Confirming the ANOVA results, the PCA analysis of chemical parameters coupled with microbiological parameters leads to clearly differentiate 5 clusters corresponding to the 5 producers.

Pathogenic bacteria were never found in Robiola samples from artisanal and industrial production, and 67 decarboxylating strains were isolated in few fresh samples, but in all ripened samples. *Enterococcus faecalis* was recognised as the most represented decarboxylating strain.

Keywords: Characterisation, microbiological and chemical parameters, biogenic amine, PDO

P014 CHARACTERIZATION OF SEMI-HARD CHEESE FROM CHIHUAHUA, MEXICO

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The impact of manufacturing protocols on the compositional and rheological properties of semi-hard Mennonite-style cheese manufactured in Chihuahua, Mexico, were determined to characterize this cheese. Cheese samples and manufacturing protocols were obtained from 11 different cheese manufacturers. Nine manufacturers made raw milk cheeses and two made pasteurized milk cheeses. Cheeses were tested at 10 days after manufacture because this style of cheese is usually consumed within two weeks of manufacture. Rheological properties were measured using small amplitude oscillatory strain, torsion, and texture profile analyses. SDS-polyacrylamide gel electrophoresis was used to determine protein and peptide profiles.

As expected, cheese composition and rheology varied among the manufacturers. Cheese composition ranged from 390 to 430 g/kg moisture, 270 to 350 g/kg fat, and 220 to 270 g/kg protein, with up to 10 % proteolysis of the α_{S1}-casein. Variations in make procedure resulted in differences in cheese composition and rheology; moisture content correlated (r = -0.86) with cooking temperature (38–45 °C); concentration of intact α_{S1}-casein correlated (r = –0.83) with time required to drain the whey (5–30 minutes); and hardness, shear stress, and shear rigidity correlated (r = 0.84, 0.90, and 0.94, respectively) with pressing pressure (0.12–0.55 MPa). Total aerobic and anaerobic plate counts ranged from $\log_{10}$ 7 to 9 cfu/g for raw milk cheeses (no starter cultures used) and $\log_{10}$ 3 to 8 cfu/g for pasteurized milk cheeses (starter culture added). Pasteurization of the cheesemilk and use of a starter culture resulted in cheeses that were significantly harder, more rigid, and less springy. Mexican Mennonite-style cheeses are defined within certain rheological ranges that identify them as

different from other cheeses such as Cheddar or brick. Establishing the basic chemical and physical properties of Mennonite-style cheese as a function of cheesemaking techniques is essential to ensuring consistent quality.

Keywords: Hispanic-style cheese, aging, rheology, proteolysis

P015 INFLUENCE OF PASTEURISATION AND MICROFILTRATION ON THE MICROBIOLOGY OF GOAT'S MILK CHEESE

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Portugal, as well as other Mediterranean countries, still maintains a significant volume of manufacture of traditional, raw milk cheeses. However, awareness of food safety issues has enforced a need to control the microbial population in such raw milk so that it may offer as high a quality as possible in attempts to guarantee the safety of the final cheese product for the consumer. Heat treatment is generally used to perform such control; however, due to attain that it may promote in the milk components, microfiltration has alternatively been shown to be another option for cheese manufacture. This study was thus carried out to evaluate the effects of different pre-treatments of raw goat's milk on the microbiological properties of cheese manufactured therefrom. A batch of goat's raw milk was divided into three equal portions: the first was pasteurised (72 °C; 50 s) (PM); the second was defatted, micro filtered (1.4 µm Membralox GP™ multi-channel ceramic membrane) and finally mixed with pasteurised cream (65 °C; 30 min) (MFM+PC); and the third was untreated raw milk (RM). Two batches of cheeses were manufactured from each batch of treated milk using a mesophilic starter culture, and ripened for 45 days at 12 °C. Duplicate samples from each milk, cream, retentate and cheeses, taken at 1, 7, 15, 21, 28 and 45 days of ripening, were assayed for viable counts of *Listeria, Salmonella,* mesophilic bacteria*,* coliforms*, Enterococcus, Pseudomonas, Lactobacillus, Streptococcus* and *Staphylococcus*.

Listeria and *Salmonella* were not detected in all assayed samples. Microfiltration was able to effectively reduce the viable numbers of coliforms to levels similar to those attained in the pasteurisation process. Viable numbers of lactobacilli were further reduced in MFM+PC than in PM milk.

Keywords: Microbiology, microfiltration, goat's milk, cheese

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P016 IMMUNOMODULATION ACTIVITY OF CHEESES FROM RHÔNE-ALPES REGION: DEVELOPMENT OF A SCREENING METHODOLOGY ON HYBRIDOMA AND HUMAN T-LYMPHOCYTE CELL LINES

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It is well known that milk-derived products such as yogurt and fermented milk contain peptides having an impact on proliferation of immune cells. Although few studies have been performed on cheeses, they represent an important potential of bioactive peptides production particularly during the ripening period. This methodology was developed using BrdU and MTT assay to assess the effect of 4 cheese water soluble extracts (Emmental, Beaufort, Abondance, Tomme de Savoie) on the stimulation of two cell lines i.e. a hybridoma (Rami cells) and a human T-lymphocyte (Jurkat cells). To quantify Rami and Jurkat growth, two associated parameters were followed: DNA synthesis by incorporation of a thymidine analogue (BrdU assay) and metabolic activity by the reduction of a tetrazolium salt (MTT assay). These tests, developed in microplates and applied to 4 cheese water soluble extracts are sensitive and allowed to screen a large number of samples simultaneously. The results we obtained show that cheese extracts hadn't the same effect depending on the cell lines and culture conditions. Extracts of Emmental and Beaufort stimulated the growth of Rami cells only when they were under a stress condition while Abondance and Tomme de Savoie stimulated the growth of these cells under all culture conditions tested. Unlike Rami cell line, Jurkat cells were strongly stimulated with extracts of the 4 cheeses.

Keywords: immunomodulation, bioactive components, cheese, hybridoma, lymphocytes

P017 DETERMINATION OF AROMA COMPOUNDS IN BITTO CHEESE BY STIR BAR SORPTIVE EXTRACTION-THERMAL DESORPTION-CAPILLARY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The instrumental characterisation of dairy products is important not only for defining cheese composition, but also for linking it with its typical organoleptic features and original environment, as required for a protected designation origin (PDO). The extraction of aroma compounds in dairy products is a complex problem due to the complexity of the matrix.

Stir Bar Sorptive Extraction (SBSE), a recently introduced solventless extraction technique, was applied in this work for the analysis of aroma compounds of Bitto cheese (typical Italian DOP cheese). This cheese is produced from June to September, when local cows and goats are maintained on alpine pasture.

A small stir bar, (10 mm length) coated with polydimethylsiloxane, (0.5 mm PDMS film thickness) was placed either in the cheese samples diluted with LC-grade water or in the headspace (HSSE) and stirred for about 1 hours.

After extraction stir bar was thermally desorbed at 200 °C for 5 min and the aroma compounds were cold trapped in the PTV inlet (Programmable Temperature Vaporizing) at –150 °C. The isolated substances were then analysed using gas chromatography-mass spectrometry (GC-MS).

The major flavour components isolated were aldehydes and ketones followed by free fatty acids and esters. Ethyl esters, in particular, play an important role in the formation of the fruity character in cheese and they are able to give this main note to the flavour of some Italian cheeses. Some terpenes such as α-pinene, caryophyllene, α-terpineol and limonene were detected. The occurrence of these compounds in cheese samples may be related to the diet of cows grazing in high mountain pastures.

This method offers the advantage of minimizing thermal, mechanical and chemical modification of the matrix, thereby reducing the risk of analytical artefacts.

Keywords: Stir Bar Sorpitve extraction (SBSE); Aroma compounds; Bitto cheese

P018 ISOLATION OF TERPENES AND AROMA COMPOUNDS IN MOUNTAIN HERBS AND TYPICAL ITALIAN GOAT CHEESE BY SBSE AND TD-GC/MSD

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Breeding of dairy goats in Italy represents a marginal rural activity. Italian goats are reared in areas where no other farming activities could be performed, therefore these animals represent an alternative in overworking the poorest mountain Alps pastures.

Mountain cheeses are still being produced today in much the same way as in the past and they are made only during summer period when goats are reared on alpine pastures. The production of their milk is quite limited, for this reason these cheeses are highly valued dairy products.

A new sampling technique, Stir Bar Sorptive Extraction (SBSE), was applied in this work for analysis of terpenes and aroma compounds in several pasture essences (i.e.: *Rumex acetosella*; *Betula alba*; *Rosa canina*; *Corylus avellana*) and then in typical artisanal goat cheese produced in mountain pasture, in order to determine the occurrence of terpenes in natural grassland of Alps and the transfer to dairy products.

A small stir bar, (10 mm length) coated with polydimethylsiloxane, (0.5 mm PDMS film thickness) was placed in the cheese samples diluted with LC-grade water and in the headspace of mountain herbs and stirred for about 1 hours.

After extraction PDMS stir bar was thermally desorbed at 200 °C for 5 min and the aroma compounds were cold trapped in the PTV inlet at –150 °C. The isolated compounds were then analysed using gas chromatography-mass spectrometry (GC-MS).

Several terpenes were isolated from pasture essences and cheese samples such as cymene, caryophyllene, γ-terpinene, α-pinene, γ-germacrene, thymol and δ-carene.

Aldehydes and ketones predominate in cheese samples, followed by free fatty acids and esters.

Some terpenes isolated from mountain herbs, in particular from dicotyledons mix of highland pastures, were detected also in cheese samples and could be used to link them to the sites of production.

Keywords: Stir Bar Sorptive Extraction (SBSE), Terpenes, Goat cheese

P019 COMPARISON OF FATTY ACID COMPOSITION IN CHEESE FROM COW'S, EWES' AND GOATS' MILK

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Cheese is obtained from curdled milk by removal of whey and by curd ripening in the presence of special microflora. The great abundance of cheese varieties, in many different sizes and shapes, can be classified from many viewpoints, e.g. according to milk utilized (cow, goat or ewes milk). The composition of milk fat is essentially triacylglycerols (95–96 %). Its fatty acid composition is complex and the content of unsaturated acids varies with season and fodder. The relatively high content of low molecular weight fatty acids is characteristic of milk.

Thirteen Portuguese Denomination Origin (PDO) cheeses were studied. According to the label, one goat milk cheese, two cow's milk cheese, eight ewes milk cheese and two mixed milk (goat and ewes) cheese, were analysed for total fat and fatty acid composition. The aim of the present work was to determine the profile of the major fatty acids in these cheeses. The motivation for this work was the realization that such profiling might make a useful contribution to identification of the species of origin of the type of milk used in the preparation of each cheese.

In general terms cheese from cow milk showed lower values for low molecular weight fatty acids. Cheese from goat milk had a distinctive pattern of stearic and linolenic acids. Cheese from ewes' milk presented higher values in oleic acid. Statistical analysis showed that the thirteen Portuguese Denomination Origin cheeses had characteristic fatty acid profiles that could be useful in cheese authentication.

Keywords: Fatty acid, PDO, authentication

P020 TEXTURAL CHANGES DURING RIPENING OF IRANIAN WHITE BRINED CHEESE

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Producing high quality cheese requires precise control over the factors affecting flavour, texture and aroma of the final product. Iranian pickled cheese is a brined cheese with a soft to semi-hard texture and an acidic, salty taste. It is made from sheep's, goats' and cows' milks or combinations of these and then ripened in brine for about 90 days in industrial production or up to 8 months in rural production units.

The objective of this work was to determine relationships between changes in textural and physicochemical factors in the central and external zones of the cheese during the ripening period. Moisture content, salt in moisture and the index of maturation were determined during the ripening at intervals of 15 days using standard methods. Rheological properties were determined by uniaxial compression of cylindrical samples (20mm diameter, 18mm height) in SMS Texture Analyser TA-XT2. The cross-head speed was 18mm/min and each sample was compressed up to 60 % of their original height at a temperature of 19±1°C. Data was collected with a computer and the deformability modulus, strain and stress at fracture, the hardness of the cheese and work up to fracture were measured, normalised for initial contact area. All measurements were made in triplicate and data were analysed statistically with SPSS software.

Results obtained so far indicated a better relation with parameters changing with ripening time than with parameters obtained from different zones.

Keywords: Texture, Rheology, Iranian cheese, index of maturation, brine cheese

P021 MONITORING OF VOLATILE ORGANIC COMPOUNDS DURING CHEESE RIPENING

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Determination of volatile organic compounds (VOC) in cheese can provide a representative set of chemical markers that characterize the origin of the product, process of production, seasoning and suitability for consummation. A relatively broad group of classes of compounds needs to be monitored since sample profiles must be compensated for varieties in seasonal changes of animal diet, which leads to variations of their presence in the milk. The determination of chemical composition in the production of cheeses serves multiple

functions. It provides an analytically accomplished chemical profiles to all production steps. This quality information serves as a basis for any evaluation, including identification of source, identity, and quality which could serve as scientific background for legal steps in the process of obtaining and enforcing the Protected Designation of Origin (European Council Regulation (EEC) No. 2081/92) or different safety standards.

In order to assign suitable chemical markers determination of origin, processes of production and seasoning, their presence along production chain and during cheese ripening must be monitored. Besides determination of suitable markers such experiment can give us information about transport of individual chemicals and their transformations during cheese production. Obtained knowledge can lead to changes in production technology in order to get product with desired seasoning and health properties.

Slovenian hard cheese „Nanoski sir„ under consideration for the Protected Designation of Origin (PDO, European Council Regulation (EEC) No. 2081/92) was studied. VOC profiles were determined by dynamic headspace with cryofocussing in animal feed, raw milk and cheese. VOC composition in cheese was determined every two weeks during three months ripening period.

Keywords: VOC, cheese, ripening

P022 CHARACTERIZATION OF THE MICROSTRUCTURE OF TERRINCHO EWE CHEESE

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The microstructure of Terrincho cheese – a Portuguese traditional cheese bearing a Protected Denomination of Origin, was assessed by scanning electron microscopy (SEM). Terrincho cheese was manufactured according to the traditional protocol using pre-filtered, plain raw ewe's milk of *Churra da Terra Quente* breed, without addition of starter of any other type of cultures, and coagulated with animal rennet. The cheeses were salted upon unmoulding, and placed in ripening chambers held at 10–12 °C and 88–89 % relative humidity for a period of 30 days. Cheeses were sampled at 0, 15 and 30 days. Cheese specimens were prepared for SEM by fixing small blocks of cheese (cut from freshly sampled cheeses) in formaldehyde, at room temperature for 1 month, cutting it into 1–2 mm thick slices using sharp razor blades and dehydrating in a graded ethanol series. Slices in absolute ethanol were critical point dried from carbon dioxide, mounted on SEM stubs using adhesive tabs, sputter-coated with gold, and examined using a JEOL scanning electron microscope.

The microstructure of the (semi-soft) curd of Terrincho cheese appeared as a continuous casein network possessing a spongy appearance. The various microbial populations present within the cheese were found to grow in the form of colonies, embedded in the casein network and close to the void spaces originally occupied by fat. Microbial colonies were roughly spherical, with bacteria tightly packed in the centre. A similar pattern of microbial growth was observed throughout ripening. Other inclusions were also pinpointed, namely salt crystals.

Keywords: Microstructure, Terrincho cheese, casein network, microbial growth

P023 EFFECTS OF HIGH PRESSURE HOMOGENISATION ON RIPENING PATTERNS OF CACIOTTA CHEESE

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The principal aim of this work was to compare cheeses obtained from cow milk previously subjected to high pressure homogenisation (HPH) at 1000 bar with those produced from raw and heat treated cow milk. The HPH treatment had both direct and indirect effects on cheese characteristics and their evolution during ripening.

The direct effects were principally linked to the quali- and quantitative modification of the microbial population, microstructure and water binding capacity of proteins. The indirect effects involved principally the activities of the microbial population and of the naturally occurring enzymes. The gas-chromatographic analyses of the free fatty acids, the SDS-Page profiles as well as the GC-SPME measurements of volatile compounds released during ripening evidenced that dynamic pressure can be regarded also as an useful tool to differentiate and positively characterise products obtained from the same raw material.

The results obtained indicated that the activation of proteolytic and lipolytic activities in cheeses obtained from pressurised milk could be linked to an enhancement of endogenous enzymatic activities, a shift of microbial population or to a different exposure of the proteins and lipids to enzymatic activity.

Keywords: High pressure homogenisation, Caciotta cheese, proteolysis, lipolysis.

P024 MICROBIOLOGICAL AND CHEMICAL STUDIES OF PECORINO SICILIANO CHEESE DURING RIPENING

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The evolution of microbial flora, compositional parameters and levels of proteolysis were monitored during the 90-day ripening period of 6 commercial Pecorino Siciliano cheeses, an Italian raw ewe's milk cheese with Protected Designation Origin status.

The mean moisture content of the cheeses at day 1 was 40.2 which decreased to 34.5 g/100g at the end of ripening. There was a resulting increase in the mean levels of salt-in-moisture during ripening from 5.94 to 8.86 g/100g. The mean cheese pH decreased from 5.7 to 5.36. Urea-polyacrylamide gel electrophoresis of cheese samples indicated that α_{s1} and β- casein were hydrolysed during ripening, leading to the formation of α_{s1}-CNf24-199 and further degradation products. Levels of proteolysis assayed as pH 4.6-soluble nitrogen and 70 % ethanol-soluble nitrogen increased in all cheeses during ripening.

Evolution of microflora during ripening was studied on a range of media. Viable counts (log cfu/g) of putative lactococci were 8.8 ± 0.3 at day 1, decreasing to 7.7 ± 0.4 at day 90, while enterococci decreased from 7.1 ± 0.6 to 6.8 ± 0.5 during ripening. However, mesophilic lactobacilli increased during ripening from 6.5 ± 0.7 to 7.7 ± 0.2. Viable counts of putative thermophilic lactobacilli remained low, attaining levels of 4.9 ± 1.1 after 90 days of ripening. Molecular analysis of coccus shaped bacteria to genus level indicated that ~ 70 % were enterococci, 20 % lactococci, 8 % leuconostoc, 2 % pediococci and 2 % streptococci. Similar analysis of mesophilic lactobacilli to species level indicated that 42% of the isolates were *Lactobacillus paracasei,* 35 % *Lb. plantarum,* 6 % *Lb. rhamnosus,* 3 % *Lb. pentosus*, 3 % *Lb. curvatus* and 11 % remained unidentified. The data further indicated that while *Lb. paracasei* and *Lb. plantarum* were isolated from young cheese, *Lb. paracasei* were dominant in the mature cheese.

Keywords: Pecorino Siciliano, microflora, composition, proteolysis

P025 EVALUATION OF BIOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF KASAR CHEESE „KAŞAR PEYNIRI"

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More than 1000 varieties of cheese are produced around the world. In Turkey, 40–50 cheese varieties are known, but only three of them have national and economic value: Turkish White, Kasar (like Kashkaval or Kasseri cheeses) and Tulum cheeses. Kasar (Kashar) cheese is the second most popular traditional cheese in Turkey with an annual production of about 41000 tonnes. It is classified as a hard cheese and requires a ripening period of 2 weeks at 12–16 °C and 3–10 months at 2–3 °C for characteristic structure. It is also manufactured industrially as a semi-hard hard cheese (known as fresh Kasar cheese in Turkey) and ripened for a shorter period. Kasar cheese is produced on a large scale, but it is not been well understood with respect to: (i) starter type and the ratio of microorganisms; (ii) the usage possibilities of some probiotic adjunct cultures in the production; (iii) effect of non-starter microflora on the quality of cheese; (iv) proteolysis, including the isolation and identification of peptides, and amino acid sequencing in the cheese during ripening. The individual volatile flavour compounds should be assessed by headspace GC-MS techniques. According to our knowledge, this is the first detailed review related to new trends in production techniques (such as use of soymilk in the production of cheese) and, biochemical and microbiological changes during ripening period of Kashar cheese.

The findings of of this review suggest that future research on Kashar cheese should characterize the changes in microflora, biochemistry and texture during ripening. Previous studies tended to focus on the chemical composition of Kashar cheese, and little attention was directed towards the detailed characterisation of flavour compounds, rheological and microbiological properties and their effects of the quality of the final product.

This review may help cheese producers to improve the quality of Kashar cheese, and should encourage further research to evaluate overall characteristics of the cheese at all stages of ripening.

Keywords: Kasar cheese, ripening, biochemical properties, microbiological properties

P026 BIOGENIC AMINES DURING RIPENING OF „PECORINO ABRUZZESE" CHEESE

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Pecorino is a traditional sheep' cheese manufactured in the Abruzzo region (Central Italy). In an artisanal plant were produced two experimental lots of cheeses: A, with raw milk and without starter; B, with pasteurized milk and addition of lactic acid bacteria (LAB) starter. All the biogenic amines (BA) were monitored in curd and during ripening of the cheeses (14, 30, 60 days). The evolution of single BA is reported in figure 1. At the end of ripening (60 days) very high amounts of histamine (261 mg/kg) and tyramine (179 mg/kg) were reached in batch A. Tyramine, ethylamine, putrescine and cadaverine were more aboundant in batch B, compared with batch A; in the same cheese histamine reached highest concentration at 30 days (124 mg/kg[1]) and then decreased during the last month of ripening (76 mg/kg). Many of *Enterobacteriaceae* and LAB isolates from cheeses were positive at amino acid decarboxylase qualitative tests; some of them resulted histidine positive (1.25%). Biogenic amine quantitative analyses were carried out on U.H.T. milk inoculated with isolates of coliforms, *Pseudomonas* and LAB after 3 days. Quantitative analyses only partially confirmed the qualitative data. Differences in the BA production were observed, depending on the microbial group or the isolate.

Keywords: sheep cheese, food safety, biogenic amines, microbiota

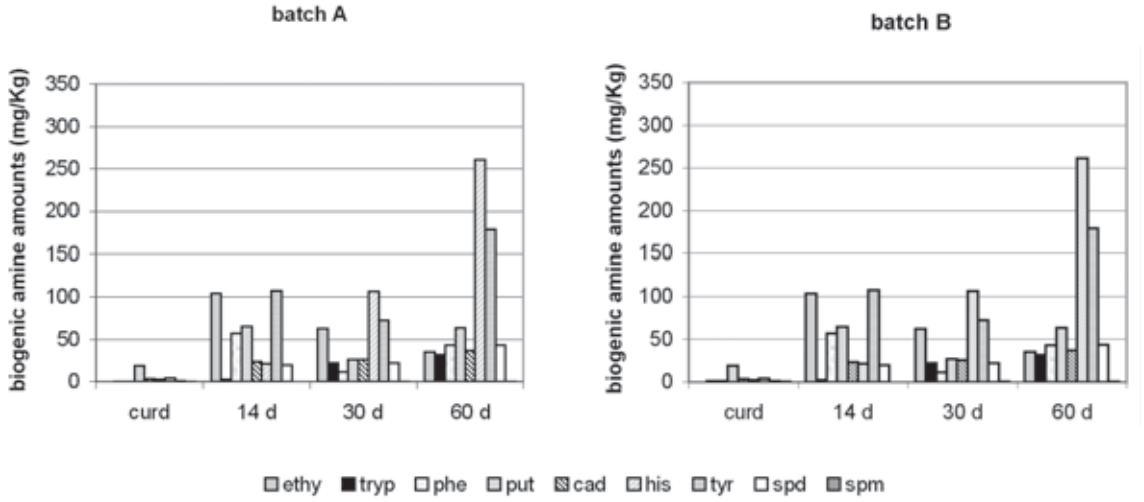

Figure 1. Biogenic amine amounts during ripening (mg/kg) in „Pecorino Abruzzese" cheese manufactured with raw milk and without starter (batch A) and with pasteurized milk and addition of lactic acid bacteria starter (batch B).
Legend: ethylamine (ethy), tryptamyne (tryp), 2-phenylethylamine (phe), putrescine (put), cadaverine (cad), histamine (his), tyramine (tyr), spermidine (spd), spermine (spm)

P027 PROTEOME ANALYSIS OF PROTEINS IN RENNET WHEY AND CASEIN COAGULUM

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The present study is part of a project involving proteome analysis to investigate the effect of milk protein composition on the yield and quality of low-fat yellow cheese. Protein content in milk from healthy, individual cows in mid lactation varies by more than 1 %, but it is not known whether this reflects significant differences in protein composition. The first step of the investigations has been to separate and characterize individual proteins in raw milk used for model cheese production. Whey protein fraction and casein curd was prepared by addition of rennet, and then separated by two-dimensional gel electrophoresis (2DGE). 2DGE separates proteins according to their isoelectrical point in the first dimension and their molecular mass in the second dimension. After 2DGE protein spots can be identified by MALDI-TOF mass spectrometry, which can also be used to characterize different variants of casein and whey proteins.

The caseins and most of the whey proteins were separated using a pH gradient of 4–7 in the first dimension. By image analysis 280 protein spots were identified in rennet whey. In order to estimate intra- and inter sample variation in 2DGE identical and parallel samples of proteins prepared by separation of tank milk were analysed and compared by image analysis. Furthermore, a range of protein spots from rennet whey and casein curd are being identified by MALDI-TOF, presently including α_{s1} casein, fragments of β-casein, BSA,

NPC2/EPV20, isocitrate dehydrogenase, α-actin, monocyte antigen CD14, transferrin and apolipoprotein A1. The study shows that proteome analysis can successfully be applied for separation and identification of proteins from bovine milk.

Keywords: proteome analysis, rennet whey, casein coagulum, raw milk, mass spectrometry

P028 SENSORIAL PROFILES OF MONTASIO, A TYPICAL SEMI-HARD ITALIAN CHEESE

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Sensorial analysis has become widely recognised as a useful tool for defining, amongst other parameters, the typical characteristics of Protected Designation of Origin (PDO) dairy products. The objective of this work was to build up a reference sensorial profile for Montasio PDO cheese, using the methodological approach proposed for hard and semi-hard cheeses in two European guides. The results obtained after two years of work have allowed the characteristics of this product, matured for 60 and 150 days, to be defined as regards intensity of smell and aroma, structural parameters (adhesion, friability, deformability, hardness and elasticity), trigeminal sensations and basic taste (sweet, salty, acid, bitter and pungent). We have considered also the possibility of to define the correlation between sensorial parameters and physical-chemical analysis. For this reason chemical determinations of sodium chloride, pH, acidity and water soluble nitrogen/total nitrogen ratio was carried out by A.O.A.C. methods, while the texture parameters were evaluated using the force-deformation curves obtained from textural profile analysis test (TPA) performed by an Instron Universal Testing Machine. Particularly, concerning structural parameters, instrumental detection of hardness, fracturability, gumminess and chewiness was correlated with casein proteolysis (water soluble nitrogen/total nitrogen ratio) and with the extend and dimension of typical holes that, in Montasio cheese, showed a high variability as a function of small variations in technology of production or in specific microflora. This could also explain the variability of the textural behaviour of the cheeses as remarked by sensorial analysis.

Keywords: sensorial analysis, Montasio cheese, basic taste, structural parameters

P029 RIPENING PROPERTIES OF VEGETABLE FAT BASED CHEESE

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When cheese is ripening, the main process involved is the enzymatic degradation of casein by enzymes originating from milk, rennet and a bacterial culture. However, a process, which also affects the sensory properties of the cheese, is the enzymatic hydrolysis of triglycerides. This process and further conversion of the fatty acids adds to the flavour development of the cheese. When milk fat is replaced with another fat, e.g. vegetable fat, the flavour development may thus be influenced.

This work involves the study of three different fat systems in semi-hard cheese of Gouda type, and their influence on the flavour development during ripening. All fat blends have similar solid fat content curves, but different fatty acid profile and crystallisation behaviour. One fat phase in the study was milk fat and one was a vegetable fat blend with a fatty acid composition resembling that of milk fat.

The fat systems were used in the production of semi-hard cheese by conventional methods. The cheeses produced were subjected to ripening, and a trained sensory panel thereafter evaluated the sensory characteristics. The results thus obtained were compared with a fingerprint of volatile aroma-compounds of the cheeses. A clear correlation can be observed between these results.

The results of this study show that choosing an appropriate vegetable fat blend can greatly influence the ripening properties and flavour profile of a vegetable fat based cheese.

Keyword: vegetable cheese, sensory analysis, ripening, flavour development

P030 INFLUENCE OF THE PERMEABILITY OF THE FILM ON THE SENSORY CHARACTERISTICS OF A RAW EWES' MILK CHEESE PACKAGED UNDER VACUUM

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Idiazabal cheese is made in the Basque Country and Navarra (Northern Spain) and must be aged three months before consumation. The possibility of removing air around cheese and then sealing the product inhibits growth of bacteria, mould, and yeast and extends shelf life. The permeability of the plastic barrier will determine the gaseous exchange through the film, and therefore the preservation of the product.

The objective of this study was to evaluate time course the characteristics of vacuum-packaged Idiazabal cheese as affected by the permeability of the film used.

Three films with increasing thickness (59, 90 and 160 mm) and decreasing O_2 and CO_2 permeability (177.28 and 759.75, 19.92 and 164.07, and 9.95 and 82.03 $cm^3/m^2 \times day \times atm$) were tested. Two cheese-making trials were carried out at pilot plant scale. For each trial, cheeses were vacuum-packaged after 3 and 5 months of ripening and underwent physicochemical, microbiological and sensory analysis with 6, 7 and 9 months. Three months-old cheeses packaged under vacuum were also tasted at 4 months of ripening. Not packaged cheeses were use as control.

Twenty assessors were selected and trained according to UNE-EN-87024-1. The panelists who passed tests based upon the Norms UNE-EN-87008 for matching tests, UNE-EN-87006 for detection of sensory stimulation and UNE-EN-87023 for intensity, were selected to evaluate odour intensity, elasticity, firmness, creaminess, grittiness, sweet-taste, pungent-taste, sour-taste, salty-taste, bitterness and aftertaste.

No differences between films were found when comparing cheeses vacuum-packaged with three months. Also, formation of aromatic compounds was slowed down as the intensity of the odour was clearly lower than in control cheeses. Cheeses packaged with five months of ripening preserved better the organoleptic characteristics, specially in the case of the highest barrier plastic film. However, significant differences between packaged cheese against control cheese increased in nine months-old cheeses, while differences between vacuum-packaged cheeses dissipated.

Keywords: Idiazabal, raw ewes' milk cheese, vacuum packaged, sensory, permeability

P031 THE CHEMICAL, MICROBIOLOGICAL AND SENSORY PROPERTIES OF HALLOUMI CHEESE PRODUCED FROM OVINE, CAPRINE AND BOVINE MILK

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Halloumi cheese, a traditional cheese variety of Cyprus, is made from raw ovine and caprine milk or from their mixture. In this study, Halloumi cheeses were produced from ovine, caprine and bovine milk, individually and chemical, microbiological, sensory properties of the cheese samples were examined during ripening. Duplicate cheese productions were performed. The total solids, fat, protein, salt, titratable acidity (SH), pH, ash, water soluble nitrogen, fat in dry matter and ripening degree values were determined at 1, 30, 60 and 90 days.

The milk origin affected the fat, protein, salt, pH, titratable acidity, ripening degree, fat in dry matter and the water soluble nitrogen value significantly ($P<0.05$). The ripening time had a significant effect ($P < 0.05$) on the water soluble nitrogen, titratable acidity, ripening degree and ash content.

At 90 days, the mean log counts of lactic acid bacteria, *Streptococcus salivarus* ssp. *thermophilus* and total mesophil aerobic bacteria were 6.23, 5.84, 6.92 cfu/g in cheeses from ovine milk; 6.04, 5.64, 6.94 cfu/g in cheeses from caprine milk; 6.49, 5.19, 7.21 cfu/g in cheeses from bovine milk, respectively.

In the sensory evaluations, Halloumi cheese produced from caprine milk was more preferred regarding properties of appearance, texture, flavour, odour and taste.

Keywords: Halloumi cheese; ovine, bovine and caprine milk; chemical, microbiological and sensory properties

P032 THE CHANGES IN TEXTURAL AND SENSORY PROPERTIES OF TURKISH WHITE CHEESE PRODUCED BY USING CORN OIL

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The amount and type of fat consumed is of importance to the etiology of several chronic diseases (e.g. obesity, cardiovascular diseases). As a result of better understanding of the relationship between diet and health, a significant change is observed in consumer's attitude towards low-fat and low-cholesterol food products.

In this study, changes in textural properties such as hardness, springiness, cohesiveness, chewiness and gumminess and in sensory properties such as appearance, flavour, texture and overall acceptability were examined during the ripening (1, 30, 60 and 90 days) of Turkish white cheese with reduced cholesterol content. Control samples were produced from cow milk with 3% fat and experimental ones from bovine skim milk mixed with 1 and 1.5 % of corn oil.

After the first day of ripening, the highest TPA (texture profile analysis) values were obtained in the samples produced from skimmed milk with 1 % corn oil depending upon high protein level. However these high values had no negative effects on the sensory evaluation. The value of hardness decreased significantly ($p < 0.05$) during ripening.

The highest values for appearance, texture and overall acceptability were evaluated at 1, 30 and 60 days of ripening in the samples produced from skimmed milk with 1.5 % corn oil. The same samples showed the highest flavour scores at 1 and 30 days while they were lower than the control cheeses at 60 and 90days.

Results show that use of 1 and 1.5 % corn oil instead of milk fat in the production of Turkish white cheese with reduced cholesterol has no negative effect on total acceptability of cheese during ripening period.

Keywords: Turkish white cheese, corn oil, texture, sensory

P033 OPTIMAL RIPENING PERIOD OF TOUNJ CHEESE ACCORDING TO PROTEOLYSIS AND SENSORIAL QUALITY

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Tounj cheese is considered as semi-hard, full-fat, smoked, farmhouse Croatian cheese produced from raw milk. According to its characteristics it was presumed that a new kind of cheese could be produced by using traditional technology and implementing ripening process. It was anticipated that ripened Tounj cheese would have highly desirable sensorial characteristics. Therefore the objective of this research was to follow basic proteolytic changes during Tounj cheese ripening and to ascertain the optimal ripening time according to proteolysis and sensorial qualities.

Proteolysis after 0, 14, 28, 42 and 56 days of ripening was measured with nitrogen fractions. Urea-PAGE electrophoresis and densitometric analysis of main casein fractions were applied. The sensorial qualities for different ripening time of Tounj cheese was judged by 16 consumers, using a scoring system with a highest score value of 20.

As a consequence of α_{s1}-CN degradation, α_{s1}-I, α_{s1}-II and some undefined degradation products appeared mainly due to chymosin action. The increasing value of these products was linear and significant ($P < 0.0001$). Beta-CN degradation by plasmin during cheese ripening was less extensive but also significant ($P < 0.0001$), the degradation products γ_1-, γ_2-, γ_3-CN appeared. Water-soluble nitrogen fraction increased significantly ($P < 0.0001$). Low molecular mass peptides and amino acids increased significantly ($P < 0.0001$) in the 12% trichloroacetic acid (TCA) soluble nitrogen fraction. The correlation coefficient between sensorial quality and degradation of α_{s1}-CN was negative but not statistically significant ($r = –0,25$), whereas the correlation coefficient between sensorial quality and β-CN degradation was positive ($r = 0,49$) and significant ($P < 0.05$) for flavor characteristics. Sensorial qualities were highly desirable for most of the consumers included in the experiment, and the optimal ripening time of Tounj cheese according to proteolysis and sensorial quality was determined to be 28 days.

Keywords: Tounj cheese, proteolysis, sensory, ripening

P034 EFFECT OF RAW AND PASTEURISED MILK ON ODOUR ACTIVE COMPOUNDS AND SENSORY CHARACTERISTICS OF PIACENTINU ENNESE CHEESE

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Piacentinu cheese is one of the many traditional cheeses of Sicily produced in the province of Enna. It is a pressed cheese with saffron added. Cheeses produced in different farms, using raw and pasteurised milk, were studied at different age (2, 4 and 6 months).

The objective of this study was to determine the differences in volatile odour compounds (v.o.c) and sensory characteristics of cheeses made from raw or pasteurised milk.

V.o.c. fractions were extracted using a retronasal aroma simulator and the SPME technique. Gas chromatography/olfactometry and mass spectrometry were used to identify v.o.c. A trained sensory panel evaluated the same cheeses. Data were analysed by split-plot ANOVA and multivariate techniques. Raw milk cheeses contained significantly more v.o.c than those made from pasteurised milk ($P < 0.05$). These cheeses also contained significantly more organic acids, terpenes, sulphur-containing and unidentified components ($P < 0.05$).

Sensory analysis showed that raw milk cheeses have significantly higher ($P < 0.05$) overall aroma intensity, green, floral, mushroom/earthy and roasted aromas than pasteurised milk cheeses, while pasteurised milk cheeses had significantly stronger fruity spicy and aromas and were more moist. Cheeses ripened for longer periods were significantly less yellow, had higher aroma intensity, were saltier, less sour and harder. Multivariate analyses separated cheeses by milk treatment, ripening time and in a few instances by individual farms.

Numbers of v.o.c, terpenes, organic acids, esters, sulphur-containing components and pyrazines, along with green, roasted, floral and mushroom/earthy aromas were all important in distinguishing between raw and pasteurised milk cheeses.

Textural characteristics, along with aroma intensity, buttery and fruity aromas, salty and sour taste and numbers of lactones and ketones played greater roles in separating cheeses by ripening time. Several individual v.o.c were also useful in making distinctions among the cheeses.

Keywords: Piacentinu cheese, GCO, sensory analysis

P035 INFLUENCE OF COMPOSITIONAL AND BIOCHEMICAL INDICES ON THE SENSORY QUALITY OF DOWNGRADED COMMERCIAL CHEDDAR CHEESES

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In this study, ten downgraded commercial Cheddar cheeses (8 full and 2 reduced fat cheeses) were subjected to various compositional, biochemical and sensory analyses. The cheeses were produced at different times of the year, using the same starter culture combination and ripened for differing periods (between 3 and 17 months) under similar conditions. Descriptive sensory analysis found that the cheeses differed significantly ($P < 0.05$) for 4 odour and 12 flavour attributes. The most mature cheeses, including those that were reduced in fat content, had the most intense flavours, and were described by terms such as 'rancid', 'sweaty' and 'soapy', which are qualities of free fatty acids. Compositional data highlighted variations in relation to moisture, salt, fat in dry matter and pH. Some of these compositional parameters were outside the national compositional parameters set for Cheddar cheese. The extent of proteolysis corresponded well with cheese age for both full and reduced fat cheese. In general the extent of lipolysis positively correlated with age and fat content, however when expressed on a fat basis, greater discrepancies existed in the extents of lipolysis. The ratio of short chain free fatty acids ($C_{4:0}$ to $C_{8:0}$) increased relative to medium ($C_{10:0}$ to $C_{14:0}$) and long ($C_{16:0}$ to $C_{18:1}$) chain free fatty acids with age in all cheeses. This possibly indicates the importance of lipolysis to Cheddar aroma/flavour, as mature cheese appears to contain higher portions of the volatile flavoursome fatty acids than younger cheese. Overall it appears that compositional parameters were responsible for the poor quality of the cheeses, which in turn influenced the extent of lipolysis, which also affected cheese quality.

Keywords: Cheddar cheese, sensory, chemical

P036 DEVELOPMENT OF A NUTTY SWEET FLAVOURED CHEESE UTILISING PROPIONIC ACID BACTERIA IN AN ALTERED CHEDDAR-TYPE PROCESS

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Recent reports indicate that sweet cheeses, similar to Swiss type cheese, have significant market opportunities. Such cheeses could be manufactured in Cheddar plants; however, processes associated with their manufacture such as pressing under whey, brine salting and incorporation of a hot room ripening step are not feasible in conventional Cheddar-type plants. This study was undertaken to develop a cheese using propionic acid bacteria (PAB) to impart a characteristic sweet nutty flavour in a Cheddar-type process.

Swiss-type control cheeses (S), Swiss-Cheddar hybrids (SC; utilising a lower pitch pH, cheddaring, milling and dry salting of curd) and modified Swiss-Cheddar hybrids (MSC; also incorporating a curd washing step during cooking) were manufactured in triplicate trials.

The cheeses were ripened at 9 or 12 °C for 180 days with the S cheeses undergoing an initial hot room step at 20 °C for 56 days. Counts of PAB in the MSC-12 °C cheeses were similar to those in the S cheese (~10^8 cfu/g) and significantly higher than those in the SC-12 °C and SC-9 °C cheeses (~10^6 cfu/g). Levels of propionic acid reflected PAB counts with the S and MSC-12 °C cheeses each containing ~ 5000 mg/kg. The MSC-9 °C and the SC-12 °C cheeses contained levels of ~ 2200 and ~ 1500 mg/kg respectively. Levels of proline, an amino acid also associated with sweetness, were similar in the S, MSC-9 °C and SC-12 °C cheeses (~3700 mg/kg) and higher in the MSC-12 °C cheeses (~5400 mg/kg). Descriptive sensory analysis indicated similar scores for nuttiness and sweetness in the S and MSC-12 °C cheeses.

Keywords: Cheese, sweet, Cheddar process, propionic acid bacteria

P037 IDENTIFICATION OF ROSEY/METALLIC FLAVOR IN CHEDDAR CHEESE

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Cheese is a natural product that may have many different flavors present. Cheddar cheeses may exhibit a rosey/metallic flavor that is typically detected after swallowing and leaves a lingering, and generally undesirable flavor. The objectives of this study were to identify and characterize aroma-active compounds that contribute to rosey/metallic flavor in Cheddar cheese using both instrumental and sensory techniques, which will enhance our understanding of cheese flavor chemistry.

Characteristic Cheddar cheeses were selected by descriptive sensory analysis using a trained panel (n = 8) and a defined sensory language for Cheddar cheese. Two cheeses (12 and 18 months old, respectively) with rosey/metallic flavor were selected along with two Cheddar cheeses of similar ages without rosey/metallic flavors. Samples were extracted thrice with ethyl ether followed by isolation of volatile material using solvent assisted flavor evaporation. Volatile extracts were separated into neutral/basic and acidic fractions and analyzed by gas chromatography-olfactometry with aroma extract dilution analysis. Compounds were identified by comparison of retention indices, odor properties, and GC-MS data against reference standards. Selected compounds were quantified using standard additions.

No differences were observed in the acidic fractions of rosey/metallic and non-rosey/metallic cheeses. The acid fraction of all cheeses contained acetic acid (vinegar), hexanoic acid (sweaty), and octanoic acid (sweaty), as well as the sugar degradation products maltol and furaneol. Some of the intense aroma-active compounds in the neutral basic fraction of the rosey/metallic cheeses included 2-phenyl ethanol (rosey), phenyl ethyl acetate (rosey), and phenylactetaldehyde (rosey/floral). Quantification and sensory analysis of model cheeses confirmed that increased concentrations of 2-phenyl ethanol, phenyl ethyl acetate, and phenylacetaldehyde caused rosey/metallic flavor when spiked into Cheddar cheese.

Keywords: Cheddar cheese flavor; GC-O; Sensory Analysis; Model Systems

P038 INFLUENCE OF STARTER CULTURE ON THE RIPENING OF EDAM CHEESE

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Sensory, rheology and microbiology of Edam type cheese (30 and 45 % FDM) was followed during ripening (1 to 6 month). Flora Danica Normal (CHN – 11) and Wisby Probat 505 were used as starter cultures. Dissimilarities in cheese were found in sensorial and rheological properties during ripening. Cheese produced with Flora Danica Normal culture had better sensory quality.

Cheese had better quality parameters up to the third month of ripening. If the period of maturation was extended up to six months, there were undesirable flavours, first the bitter taste and strange flavour. Hardness in mouth and hardness between fingers decreased, cohesiveness and smearness increased. 45 % FDM cheese was evaluated as softer, more smearness and more palatable.

Microbiological quality was measured during ripening (total plate count, coliform count, lactic acid bacteria, anaerobic bacteria, yeasts and moulds). Total plate count, yeasts and moulds increased in 3 month. The occurrence of cracks increased from 4 to 6 month of ripening.

Keywords: Edam cheese, sensory evaluation, rheological properties, ripening

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P039 CHEMICAL AND SENSORY QUALITY OF EVORA, A HARD EWE CHEESE

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Évora cheese is an AOP cheese farmhouse-made from raw ewe milk, in the Alentejo region of Southern Portugal. It is manufactured between the months of December and June, according milking season of the ewes. The cheese is ripened for 30 to 45 days, the texture shows a semi hard to hard consistency, the diameter is 6–7 cm and the weight 80–120 gr.

The extensive system of production of the ewes, the manufacture process and the ripening conditions provide chemical and sensorial characteristics witch contribute to the specificity of Évora cheese.

The chemical composition and the proteolysis indices of the cheese varied significantly during the ripening period, even though not similar throughout the cheese-making season.

According to the results of the volatile components, the cheese is characterised by high levels of lipolysis, compared with a low proteolysis, presenting extremely high concentrations of fatty acids, esters and ketones. It was detected volatile components that are normally associated with cheese produced from the milk of grazing ewes.

A taste panel defined the sensory profile of the cheese and the typical flavour of ewes' cheese, witch is associated with piquant, ewe and salty flavours. The examination of the results of PCA suggested that some chemical components probable could explain some specific flavour attributes.

Keywords: Ewe cheese, hard cheese, volatile components, sensory

P040 MEASUREMENT OF CHEESE TEXTURE AND OPENING BY ULTRASOUND TECHNIQUE

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In this work we compare two ultrasonic techniques applied in the frequency range of 30–70 kHz in order to observe opening and the elastic properties of the cheese at different scales (local measurement and measurement realised on the main part of the product). In this case, the study relates to cheese characterization in the refining phase presenting two opening types: cracks alone and eyes with cracks. The analysed acoustic data related at the same time on the frequency spectrum of the signal and to the time signal obtained in a transmission mode through the cheese matrix. The results are not only compared with measurements of reference on cheese, but also with the optical images obtained after cutting. The conclusions of this part of the study show the good sensitivity of the ultrasounds to the non-destructive characterization of texture of the cheese. The results obtained by acoustic measurements during the end refining phase with other frequencies make it possible to consider a fast and total characterization of the final product of cheese-making process.

Keywords: Acoustic, cheese, texture, opening, ultrasound

P041 PREDICTION OF MOISTURE AND FAT IN PROCESSED CHEESE BY NEAR INFRARED REFLECTANCE SPECTROSCOPY AND MULTIVARIATE DATA ANALYSIS

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Near infrared (NIR) spectroscopy is a rapid, easy-to-use, and non-destructive analytical technique. This paper reports on its application for the prediction of moisture and fat in processed cheese.

Processed cheese samples were manufactured with varying contents of moisture, fat and emulsifier according to an experimental design and aged for 28 days at 4 °C. After 14 and 28 days, aliquots were subject to NIR spectroscopic analysis and chemical testing for moisture and fat. NIR spectra (FOSS NIRSystems 6500 spectrophotometer) were collected in reflectance mode on cheese cylinders in a circular reflectance cup. Reference measurements were made using an air oven (moisture) and Röse-Gottlieb method (fat). Calibrations for these constituents were developed using modified partial least squares (mPLS) regression in WinISI software. A number of wavelength ranges and data pre-treatments were investigated. Best models used spectral data between 1100 and 2498 nm in both cases.

Both constituents were modelled well. Moisture (range = 37.7–54.8 %) was predicted with a standard error of cross-validation (SECV) of 0.48 % and a correlation coefficient (R) of 0.99 (5 PLS loadings). Fat (range 23.7–34.0 %) was predicted best by a 5 loading model (SECV=0.45 %; R = 0.98). In both cases, these models involved a scatter (standard normal variate and de-trend) and a 2nd derivative (10nm gap) pre-treatment.

These results suggest a role for NIR reflectance spectroscopy in the off-line quality assessment of processed cheese.

Keywords: processed cheese; near infrared spectroscopy; chemometrics; proximate analysis

P042 SPECTROCOLORIMETRY IN THE CIE LAB COLOR SPACE AS USEFUL TOOL FOR MONITORING THE RIPENING PROCESS AND QUALITY OF RED-SMEAR SOFT CHEESES

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The appearance of foodstuffs is the only permitted way to appreciate on-sell food products. In that respect, color is a clue for many qualities of food such as flavour, sanity, naturality or maturity, and drives consumers' choices. An attractive aspect is therefore a key for food marketing, and this has led the food industry to devote much effort in offering pleasant and suggestively colored products. The smeared cheeses like Limburger, Maroilles, Munster or Tilsit, are characterized by the occurrence of red to orange-brown surfaces. The red smear of these cheeses originates in the synthesis of carotenoid or other pigments by bacteria, in particular *Brevibacterium linens* and coryneforms. Objective measurement of the smear color can be provided using a spectrocolorimeter. This device mimicks the psychosensorial mechanism of human perception of color. It integrates both the light source spectrum and the object color spectrum into the reflectance spectrum. The latter is further filtered so that the red, green and blue spectra are extracted as 3 coordinates (as they are by the human eye's rods) then processed to obtain the three dimensions L*a*b* response. As a means to bring information to cheese manufacturers making red-smear soft cheeses, it was the objective of the present work to investigate the use of spectrocolorimetry for the description of: i) the relative heterogeneity observed for „on shelf cheeses" color among a PDO area (example taken from Munster produced in France), ii) the assessment of color development at the surface of cheese versus time (quality control), iii) a screening technique for pigmented strains isolated from red-smear cheese rinds (biodiversity criteria), iv) the interactions observed between the pigmented bacteria of the microflora and the yeasts used in cheese making.

Keywords: spectrocolorimetry, red-smear cheese, color, ripening

This work was supported by ACTIA (Association de coordination technique pour l'industrie agro-alimentaire) research programmes 99.14 and 02.11, the French Minister of National Education, Research and Technology (M. E. N. R. T.), Degussa France and four cheese producers.

P043 DISCRIMINATION OF EUROPEAN EMMENTAL CHEESE BY MID-INFRARED SPECTROSCOPY

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The potential of Fourier Transform mid-infrared spectroscopy (FT-MIR) was investigated to discriminate 166 Emmental cheeses produced during summer (72 samples) and winter (93 samples) from five European countries: Germany, Austria, Finland, France (Savoie, Bretagne and East-central) and Switzerland. The cheeses were produced from raw milk except the samples from Finland, Bretagne and East-central France.

The analyses were carried out on dry matter of the samples and the 2nd derivatives of the spectra, between 1050 and 1800 cm^{-1}, were calculated. The data were analysed by principal component analysis (PCA) and partial least square regression (PLS) according to the season of production, the treatment of the milk and the geographic origin. For the PLS classification, the spectra collection was split up into three sets, calibration and validation sets to create the model and a test set using others samples.

PCA showed a good discrimination of summer and winter cheeses. 85 and 81 % of the calibration and validation samples were correctly classified by PLS, respectively.

The effect of the milk heat treatment couldn't be observed by PCA. PLS showed a good classification for 93 % of the calibration and validation samples from raw and pasteurized milk. All the cheeses made from raw milk were correct classified.

The discrimination between the five production regions was weaker. 71 and 68 % of the calibration and validation samples were correct classified, respectively.

It was concluded that mid infrared spectroscopy is a promising method for the discrimination of cheeses and especially the season of manufacture and the heat treatment of the milk.

But, this method alone will not make a good separation from the geographic origin. It is very likely that it will be necessary to take into account other parameters and to combine various analytical methods to improve the discrimination.

Keywords: Authenticity, MIR spectroscopy, cheese

P044 MEASUREMENT OF GAS HOLES AND MECHANICAL OPENNESS IN CHEESE BY IMAGE ANALYSIS

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A method to measure the amount of the surface area of cheese slices occupied by gas holes was developed to reflect the relative gas production among different cheeses. A digital camera mounted on a copy stand with lighting was used to make digital images of each slice of cheese. A commercial digital image analysis software program was used and an algorithm written to measure the area of the image of the cheese slice occupied by holes.

The image was cropped and scanned to determine which color channel produced the best image contrast. The MATLAB® program allowed the user to eliminate mechanical openness or false holes and then to scan the image to produce a percent distribution of pixels in the image as a function of pixel intensity.

The user then determined a threshold value to differentiate pixels that were in holes from those representing areas with no holes. The percentage of the total surface area occupied by holes was calculated. The coefficient of variation of the method ranged from 2.43 % at a level of gas holes of about 1 % of the surface of the cheese slice to a coefficient of variation of 0.92 % at a level of gas holes of about 6.8 % of the surface area of the cheese slice.

Examples of applications of this method are given for Emmental, Ragusano and Cheddar cheeses. The method can be used as a tool in research studies to correlate the amount of gas production with manufacturing conditions or as a quality control tool in cheese manufacturing.

Keywords: image analysis, gas production, mechanical openness

P045 FT-NIR SPECTROSCOPY TO STUDY PACKED INDUSTRIAL RICOTTA DURING SHELF-LIFE

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Ricotta is a very interesting Italian dairy product, made through acid-thermal coagulation of whey and characterised by a short shelf-life.

The aim of this study was to monitor modifications during shelf-life of packed industrial ricotta by using a simple and fast method. The feasibility of FT-NIR spectroscopy was verified for its ability to evaluate the quality of Ricotta during shelf-life in relation to the evolution of chemical and rheological parameters.

Several samples of manufactured Ricotta were analysed during shelf-life at three different storage temperatures (3, 10 and 20 °C).

Spectral data were collected in a spectral range, from 12000 to 4400 cm^{-1} by using a FT-NIR spectrometer with an optic fibre working in diffuse reflectance.

Titrable acidity, NH_3, peroxide index, sugars, lactic and citric acids were determined.

Texture was measured by using both the vane method and the back-extrusion test. Spectral measurements were carried out on the original packages.

Principal Component Analysis was applied as exploratory chemometric technique to each one of the three sets of data (FT-NIR, chemical and rheological data).

Then, a calibration model between the NIR data set and some chemical and rheological indices was built by means of a PLS1 regression. The number of latent variables was optimised combining results obtained by calculating the Durbin-Watson criterion for the B-coefficients vectors, and by PLS1 cross-validation. Chemical and rheological indices were chosen on the basis of the distribution of their PCA scores.

The PCA results from each set of data, showed there was a critical day of shelf-life for each storage temperature. After this day, the product is no more acceptable. From the chemical, rheological and spectroscopic variations the same critical day was confirmed, demonstrating the relationship between the different techniques used.

Keywords: NIR spectroscopy, chemometrics, shelf-life, Ricotta

P046 USE OF ELECTRONIC NOSE AND TRAINED SENSORY PANEL IN THE EVALUATION OF SERPA CHEESE

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Serpa cheese is a soft creamy paste cheese, with AOP designation, obtained from the curdling of raw ewe's milk, by the action of an infusion of *Cynara cardunculus L.* The cheese was produced traditionally with ewe's milk from local breeds, Merino and Campaniça, raised under extensive range conditions. This is a traditional dairy product having high intrinsic value, arising from their unique sensory characteristics, coupled with long-recognized social and economic impacts.

Serpa cheese is manufactured without any starter addition, so the microbiological composition of young cheese is strictly dependent on the microbiological quality of the milk. The variability of milk composition, differences in manufacturing processes and the different ripening conditions were source of cheeses heterogeneity, follow-on different cheese quality among the cheese makers.

Trying to evaluate the scope of the different cheese quality presented in a typical fair, in the Alentejo region, AOP cheeses manufactured by seven different cheese makers were tasted by the trained official Serpa cheese sensory panel and results were compared to the spectrum obtained by the electronic nose detection. The electronic sensory instrument is especially designed to characterize a global aroma pattern trying to mime the results obtained with the human nose. This technology allows discrimination between samples, acceptability prediction that improves the relationship between consumers and producers.

The results presented indicate that the best cheeses (Good cheeses) according to the trained sensory panel were grouped when analysed by the electronic nose, and were clearly separated from those negatively ranked by the panel (Bad cheeses).

Under these conditions it is possible to establish a Good/Bad cheese standard using the electronic sensory analysis. So the quality assessment of unknown samples of Serpa cheese can be predicted, using this instrumental analysis, with a certain high level of confidence.

Keywords: Electronic nose, sensory panel, Serpa cheese

P047 TEXTURE AND PROTEOLYSIS PATTERN IN A CHEESE MODEL: STARTER-FREE, FULLY CONCENTRATED, RENNETED SKIM MILK MF RETENTATE

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Diafiltered skim milk MF retentate was through the addition of rennet, NaCl and GDL converted into a cheese model. No whey was released and thus the exactly amounts of rennet and whey proteins are known. Bacterial flora was totally suppressed with sodium azide and penicillin.

Samples were stored at 12 °C and analysed at ages up to 1 month by uniaxial compression, capillary electrophoresis (CE), colour measurements, calcium and phosphorus in the water phase (ICP) and transmission electron microscopy (TEM).

Even at constant casein concentration, the textures could be manipulated from stiff/elastic to soft. At high pH and low rennet, the samples did not change during storage and had an apparent compression modulus at fracture of several Mpa. At low pH, high rennet content and high salt content, the samples were soft and turned pasty, not self-supporting, within a few weeks.

The model offers the opportunity to elucidate the specific role of the proteolytic activities of plasmin and chymosin in the development of microstructure and textural properties of cheese during storage.

Keywords: texture development, microfiltration retentate, cheese model

P048 UTILIZATION OF DIFFERENT STARTERS FOR THE MANUFACTURE OF MOZZARELLA CHEESE

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Different ratios of coccus to rod in starter cultures were used for the manufacture of Mozzarella cheese. Chemical composition, rheological and organoleptic properties of Mozzarella cheese were determined. The coccus to rod ratio did not affect the gross composition of cheese. Control cheese (*Streptococcus salvarius spp. thermophilus* + *Lactobacillus delbruecki spp. bulgaricus* 1:1) showed the highest firmness and concentration of galactose whereas, lowest concentration of galactose was found in the cheese made with *S. thermophilus* + *L. helveticus* (1:10). The highest browning intensity in the heating test appeared in cheese manufactured with *S. thermophilus* + *L. bulgaricus* (1:1). While the lowest degree of browning was found in cheese manufactured with *S. thermophilus* + *L. helveticus* (1:10). Slab gel electrophoresis pattern of proteins for Mozzarella cheese stored 4 weeks at 4 °C showed more proteolysis in cheese made with low coccus: rod ratio than the other treatment.

Keywords: Mozzarella cheese, starter culture

P049 RELATION BETWEEN PROTEOLYSIS AND QUALITY DEFECTS DURING RIPENING OF SWISS CHEESE

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Twenty-eight Swiss type cheeses were manufactured using the standard method in the pilot equipment of Dairy research institute. Samples were taken at 1, 15, 45, 75 and 120 days for chemical and microbiological analysis and sensory evaluation.

After 120 days the cheeses were classified by their quality into three groups. No or only few splits occurred in the best products (group A – containing 10 cheeses). In the second transition group B of 8 cheeses has appeared only lower count of these defects. In the third group C of 10 cheese were observed significant defects (splits and undesirable nutty eyes (holes) formation) on the cheese cut and higher occurrence of sensory defects (bitterness, off-flavour).

Significant differences comparing cheese groups A and C were found in proteolysis during ripening. These were described by LN pH 4.6, TCA-SN and PTA-N content, by differences in lower carboxylic organic acids (acetic and propionic acid) and by count of determinated microorganisms groups (proteolytic and psychrotrophic bacteria in cheese milk and thermophilic microorganisms during cheese ripening). The results show that on the level of cheese proteolysis at 45 days it is possible to determinate the activities of presented enzymatic systems and predict the occurrence of split defects in cheese at 120 days.

Keywords: Proteolysis, Swiss cheese, quality defects

P050 A MODEL OF SWISS-TYPE CHEESE RIPENING

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A cascade model of Swiss-type cheese ripening was postulated. We assume that during coagulation, milk fat globules and bacteria are randomly entrapped into the continuous network of casein micelles.

Casein hydrolysis starts with formation of N-terminal peptides by action of plasmin inside the casein micelles. The N-terminal peptides formed can diffuse to the colonies of starter bacteria. The proteolytic enzymes of starter colonies degrade peptides to amino acids, which are able to reach the colonies of NSLAB and support their growth.

The growth and autolysis rates were calculated in the framework of the solid state grid model and juxtaposed with the experimental data on the proteolysis of casein. The autolysis was initialized during salting and led to lysis of most starter cells in their colonies corresponding to the release of endopeptidases.

Using PAGE data and taking into account the primary structure of caseins and specificity of proteinases a quantitative scheme of the primary proteolysis of casein was developed. 37% α_{s1}-casein remained intact during the whole process of ripening but α_{s1}-I casein (24-199) – the peptide formed during the primary proteolysis was not degraded. About half of β-casein (47%) remained intact and γ-caseins formed were hydrolyzed further.

Comparison of the experimentally measured and calculated data showed that the quantities of the amino acids available from the N-terminal peptides were sufficient to formation of free amino acids found in cheese during first month of ripening. We assume that afterwards most of the β-casein, molecule except the region 120–183, is also degraded.

Cascade model agreed quantitatively with our experimental data on bacterial growth and casein hydrolysis.

Keywords: Swiss-type cheese, proteolysis, ripening model

P051 PURIFICATION AND IDENTIFICATION OF PHOSPHOPEPTIDES FROM CHEESE USING FE(III) AFFINITY CHROMATOGRAPHY AND MASS SPECTROMETRY

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One of the important nutritional aspects of cheese is the high content of organic phosphate associated with calcium. Digestion of phosphorylated peptides and proteins facilitates uptake of calcium and thus contributes positively to stronger teeth and bone. The composition of phosphopeptides may also influence consistency of low-fat semi-hard cheese. Phosphopeptides are preferably isolated from cheese through interaction with polyvalent metal ions either by precipitation or chromatographic separation. In present work, water soluble phosphopeptides from cheese were isolated using immobilized metal affinity chromatography (IMAC). An optimized method based on immobilization of Fe(III) on IDA-Sepharose was developed. Water soluble phosphopeptides from cheese were quantitatively retained on the IDA-Fe(III) column and could be eluted with ammonium phosphate. Phosphopeptides up to 2500 D in the eluate from the IDA-Fe(III) column were subsequently identified using reversed phase LC-ESI tandem mass spectrometry.

Two different cheeses were characterised using the described method: a 10 weeks old Herrgĺrd cheese made with mesophilic DL-starter and a 24 month old Parmagiano Regiano cheese made with thermophilic starter. The two cheeses have a highly different distribution between organic bound phosphorous and inorganic phosphorous. Of the total phosphorous found in aqueous extracts, organic bound phosphorous in Herrgård and Parmagiano Regiano accounted for 84 % and 20 %, respectively. Elution of the IDA-Fe(III) column with a gradient of 0.2 M ammonium phosphate resulted in three major peaks corresponding to peptides carrying one, two, and three phosphorylated serins, respectively. Considerably higher amounts and a higher number of different phosphopeptides were found in Herrgĺrd cheese than in Parmagiano Regiano cheese.

Keywords: Cheese, phoshopeptides, metal affinity chromatography, mass spectrometry

P052 EQUILIBRIUM OF WATER-SOLUBLE N OF FETA CHEESE BETWEEN THE CHEESE BLOCKS AND THE BRINE

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It was observed that proteolysis in Feta cheese (and the other cheeses in brine) cannot be precisely estimated by determination of the water-soluble N, because a significant part of this fraction – at least at the early stages of maturation – consists of serum proteins (SP). In the RP-HPLC chromatograms of the water extract (WE) of a 3-d old Feta cheese (before the cheese was placed in the brine) the peak area of SP was about 25 % of the total chromatogram area.

Later on, as the cheese matures in brine, both the soluble casein fragments and the SP diffuse to some extent into the brine. The RP-HPLC profiles of the WE of Feta cheese at various ages showed a gradual decrease of size of the SP peaks. Already at 20 d, an average 40 % decrease of the peak area was estimated.

The diffusion of peptides and SP into the brine was confirmed by RP-HPLC analysis of the brine itself. The peak area corresponding to the SP and to the peptides eluted up to 33 % (v/v) acetonitrile continuously increased. However, very few peaks were eluted with acetonitrile concentrations higher than 33 % (v/v) with the exception of SP. It was therefore assumed that the diffusion of peptides was related to their hydrophobicity and size. To confirm this hypothesis, water extracts of Feta were treated with TCA and the supernatants were also analysed by RP-HPLC. The profile of the 12 % (w/v) TCA soluble N was similar to that of the brine. Concerning the SP, their diffusing aptitude could be attributed to their mean molecular hydrophobicity (1082 cal/res).

Keywords: Feta cheese, brine, serum proteins, peptides

P053 RIPENING CHARACTERISTICS OF FORTIFIED TURKISH WHITE CHEESE

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Turkish white cheese (TWC) ripened in brine is a kind of semi-soft one with slightly acid and salty flavor. It may be consumed in fresh or after ripening.

In this study, the fortification effects on the level of ripening of zinc (Zn) and selenium (Se) in TWC produced for some risk groups were examined. The fortification degrees of zinc and selenium were calculated by taking the needs of daily intake of those risk groups into account (80 mg/kg for zinc, and 0.37 mg/kg for selenium). For these purposes, the milk to be used for white cheese production was added with 160 mg/kg of zinc and 0.73 mg/kg of selenium. Standard production technique was followed in white cheese production. The amounts of recovering of zinc and selenium in white cheese samples were found 87.5 and 58.1 %, respectively.

The levels of proteolysis in cheese samples stored at +4 °C were analyzed by using the water-soluble nitrogen (WSN), trichloroacetic acid-soluble nitrogen (TCA-SN) and the reverse-phase HPLC techniques during the 1, 30 and 60 days of the storage. Increases were observed in WSN and TCA-SN during period of ripening. According to the control samples, the effects of fortification were considered significant on WSN of TWC ($p<0.05$) and not significant on TCA-SN ($p>0.05$). It was determined that fortification particularly caused accumulation in hydrophobic region despite the HPLC peptide profiles of water-soluble nitrogen material were rather similar.

Keywords: Turkish white cheese, zinc, selenium, fortification, proteolysis, HPLC

P054 LEVELS OF PROTEOLYSIS IN IMPORTANT TYPES OF TURKISH CHEESES

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In 2001, 9'495'554 tons of milk were produced in Turkey out of which 43 % was transformed into cheese, 25 % into fermented milk products (yogurt, ayran, etc.), 17 % into butter and 16 % was consumed as fluid milk. As it is evident, close to half of total milk production was processed into cheese, thus placing this product amongst the exceptional and important Turkish milk products. Approximately 50 % of cheese milk was used for Beyaz Cheese (White Pickled Cheese) and Kasar Cheese. The rest was used to produce other traditional Turkish cheeses like Tulum, Mihalic, Dil, Oltu, Cokelek, Civil, Comlek etc. Amongst these traditional Turkish cheeses Tulum Cheese is the most important and it is ranked third in terms of production and consumption quantity after Beyaz and Kasar cheeses.

Whereas, Beyaz Cheese is a semi-hard ripened cheese and has a slightly salty and acid taste, Kasar is a hard-ripened cheese belonging to the „kneaded cheese" type and it has a piquant flavor. Tulum Cheese is a hard cheese ripened in a goatskin and has a piquant, rancid and a sharp flavor.

This poster describes the results of the studies conducted to determine the levels of proteolysis of Beyaz, Kasar and Tulum cheeses, on 124 samples (40 Beyaz, 42 Kasar and 42 Tulum.) The average amounts of total nitrogen (TN), water soluble nitrogen (ripening coefficient), 12 % TCA soluble nitrogen, proteose peptone nitrogen, phosphotungstic acid soluble nitrogen of Beyaz cheeses were 2.347, 0.490 (20.74 % of TN), 0.285, 0.204, 0.111 g/100g, respectively. The same properties of the Kasar cheeses were 4.174, 0.523 (12.48 % of TN), 0.280, 0.243 and 0.117 g/100g, respectively. The concentrations of the same characteristics in the Tulum cheeses were 3.416, 0.598, (17.61 % of TN), 0.444, 0.155 and 0.239 g/100g, respectively.

Proteolysis levels show that the three main types of cheeses are consumed as ripened cheese. Similar levels of proteolysis were found in unripened cheese, which is indicative that most cheeses are not stored by their manufacturers for prolonged periods of time until their maturity.

Keywords: proteolysis, Turkish, cheese, ripening

P055 THE EFFECT OF BACTERIOCIN-PRODUCING *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* INIA 415 AND ITS BAC-MUTANT INIA 415-2 ON HISPÁNICO CHEESE PROTEOLYSIS AND TEXTURE

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Hispánico cheese is made in Spain from a mixture of pasteurized cows' and ewes' milk. In the present work, a bacteriocin-producing adjunct culture was used to enhance starter lysis in cheese to accelerate the ripening process.

Duplicate experiments were carried out, each consisting of two vats containing 40 L cows' milk and 10 L ewes' milk. Lactic cultures for experimental cheese were 0.5 % *Lactococcus lactis* subsp. *lactis* INIA 415, a Bac+ strain harbouring the structural genes of nisin Z and lacticin 481, 0.5 % *L. lactis* subsp. *lactis* INIA 415-2, a Bac- mutant and 2 % TA052, a commercial *Streptococcus thermophilus* culture. Lactic cultures for control cheese were 1 % *L. lactis* subsp. *lactis* INIA 415-2 and 2 % TA052. Cheeses were pressed, brine-salted and ripened at 12ȘC for 75 days.

Milk inoculation with the bacteriocin producer lowered *S. thermophilus* counts in cheese and enhanced the release of intracellular peptidases, indicating early lysis of the thermophilic culture. Proteolysis of experimental cheese, estimated by the *o*-phthaldialdehyde method, was 1.38-fold that of control cheese on day 25 and 1.49-fold on day 75. A higher increase was recorded for total free amino acids, with levels in experimental cheese 2.47-fold that of control cheese on day 25 and 2.34-fold on day 75.

Levels of residual a-casein were significantly higher in experimental than in control cheese throughout ripening, whereas levels of β-casein were higher in experimental cheese on day 1 but did not differ afterwards. Levels of both hydrophilic and hydrophobic peptides were higher in control cheese than in experimental cheese throughout ripening.

Higher values of fracturability, elasticity and hardness were recorded on days 50 and 75 for cheese made with the bacteriocin producer. The firmer texture of experimental cheese was related to its higher level of residual α-casein.

Keywords: cheese, bacteriocin, proteolysis, texture

P056 ISOLATION AND IDENTIFICATION OF SOME MAJOR PEPTIDES IN THE 70 % ETHANOL-SOLUBLE FRACTION OF MANCHEGO CHEESE

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Proteolysis is one of the major biochemical events which takes place during cheese ripening and the products of proteolysis, amino acids and peptides, have a considerable influence on the sensory characteristics of cheese. Primary proteolysis leads to the formation of large water-insoluble peptides and smaller water-soluble peptides. The identification of peptides from cheeses made from bovine milk has been well studied. However, there is little known of the identity of peptides from ewes' milk cheeses.

The objective of this work was to isolate and identify peptides in the 70 % ethanol-soluble fraction of Manchego cheese, in order to understand better the proteolytic pathways during the ripening of this cheese which is made from ovine milk.

Peptide profiles of the ethanol-soluble fractions of pH 4.6-soluble extracts were determined by RP-HPLC. A total of 14 peptides were identified. The peptides contained between 6 and 17 amino acid residues and most of them originated from the N-terminal domain

of α_{s1}-CN, whereas only two of them corresponded to the C-terminal domain of β-CN. Most of the sites cleaved to yield the peptides identified in this study have been found to be cleaved in other cheeses.

Keywords: Manchego cheese, ethanol-soluble peptides

P057 EVALUATION OF PROTEOLYSIS IN PDO RAGUSANO CHEESE DURING RIPENING BY CHEMOMETRIC ANALYSIS OF PEPTIDE AND FREE AMINO ACID PROFILES

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Proteolysis is essential for flavour and texture development in cheese. Chemometric analysis of proteolytic profiles can assist in better understanding proteolysis and the factors that influence it. Multivariate analysis of peptide and free amino acid (FAA) data has been successfully applied in discriminating among cheese varieties, quality and age or for detecting fraudulent products.

Chemometrics was applied to the study of proteolysis in PDO Ragusano cheese during ripening. Cheeses were sampled at 3, 4, 6 and 7 months and four layers were analysed (rind, external, middle and core).

Urea-PAGE profiles showed increasing hydrolysis of caseins from the cheese surface to the core and throughout ripening. Levels of pH 4.6-soluble N were significantly affected by cheese layer and age. Increasing levels of 70 % ethanol-soluble and -insoluble peptides were found by RP-HPLC in the innermost cheese layers and as the cheeses aged. Similar, but non-significant, trends were found for non-protein N. Increasing levels of individual FAA with cheese age and slice depth were not significant except for Asp. Glu, Lys and Leu were the predominant FAA (45 % of total FAA). Total FAA ranged from 17.6 mg/g (6.5 % of total protein) at 3 mo to 26.1 mg/g (8.9 % of total protein) after 7 mo.

Principal Components Analysis and Hierarchical Cluster Analysis of the data from RP-HPLC and FAA analyses showed that the peptide profiles were most useful in differentiating Ragusano cheese by age and farm origin. Combining soluble and insoluble peptide data resulted in an excellent Partial Least Squares (PLS) Regression model ($R^2 = 0.961$; $Q^2 = 0.923$) predicting cheese age. The 20 most important predictors were soluble and insoluble peptides with medium hydrophobicity. This model also resulted in a 100 % correct classification by PLS Discriminant Analysis of cheeses according to age. Hydrophobic peptides were discriminatory for distinguishing among sample classes.

Keywords: Ragusano cheese, peptides, FAA, chemometrics

P058 MODIFICATIONS IN CHEESE TEXTURE AFTER HIGH PRESSURE PROCESSING TREATMENT

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High Pressure Processing (HPP) has been proposed as an alternative technology for preserving food. HPP has already been applied in the dairy field to reduce cheese ripening and to increase cheese yield.

In the last decade, a lot of studies have been made in order to investigate the effects of HPP on chemical and physical composition of milk and milk products. In particular, the influence of HPP on texture of Gouda, Mozzarella and goats' milk cheese has been studied.

The purpose of this work was to investigate if HPP applied to ripe products can affect the original structure of cheese.

Different types of cheese, such as Grana Padano, Emmental, Caciotta, Quartirolo and Pannerone, were processed and tested with an Instron™ (Instron Ltd., High Wycombe, UK) Universal Test Machine, before and after application of the HPP treatment using uniaxial compression.

The texture of all different types of cheese was affected by HPP, but the modifications differed according to the type of cheese.

There was a completely different behaviour particularly in Grana Padano cheese, which is a hard and crumbly cheese.

Keywords: high pressure processing, cheese, texture, uniaxial compression

P059 QUALITY CONTROL OF RENNETS AND CHEESES USING ELECTROPHORETIC AND CHROMATOGRAPHIC METHODS

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Calf chymosin is still the prevalent milk coagulant used in cheesemaking, but due to the shortage of calf stomachs, animal rennets are sometimes fortified by the addition of pepsins. Furthermore, proteolytic enzymes of microbial origin are widely used for cheese manufacturing, and recombinant calf chymosin produced in micro-organisms is now also in industrial production. In some countries, vegetable rennets are traditionally used, but compared to animal and fungal rennets, vegetable and bacterial proteases with milk-clotting properties play a negligible role in cheese industry.

In Austria, an important private company had produced rennet of animal origin for a long time according to a well-established procedure. Recently, they changed their technological process to produce an improved quality of a traditional type of calf chymosin (no recombinant rennet).

A comprehensive quality control programme included the characterisation of the old and new rennet preparation themselves, as well as the chemical evaluation of different types of cheese (e.g., Camembert, Edam cheese, Bergkäse), which had been produced in cheesemaking experiments using the old and the new product, respectively.

Using urea-PAGE, SDS-PAGE and isoelectric focusing as well as reversed phase-HPLC, significant differences between the old and the new rennet could be observed indicating that the new purification process resulted in a rennet preparation of much higher purity.

Proteolytic changes in cheese samples of different types and ripening ages were studied using electrophoresis and RP-HPLC of caseins as well as of nitrogen fractions soluble in water and 50 % ethanol, respectively. Using these indices of proteolysis during ripening, no significant differences could be found between the cheeses, which had been produced using the old and the new rennet preparation, respectively.

Thus, electrophoretic and chromatographic analyses of caseins and soluble nitrogen fractions of cheeses proved to be appropriate tools for the quality control of rennets and for the routine evaluation of proteolysis in cheeses during the ripening period to guarantee the high quality of dairy foods.

Keywords: cheese, rennet, electrophoresis, RP-HPLC

P060 „LAB-ON-A-CHIP" TECHNOLOGY FOR THE MEASUREMENT OF PROTEOLYSIS IN CHEESE

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A variety of different analytical methods, including electrophoretic (SDS-PAGE, urea-PAGE, capillary electrophoresis) and chromatographic (gel permeation, ion exchange, reversed phase HPLC) have been used to measure proteolysis during cheese ripening.

Traditional slab gel electrophoretic techniques have been shown to be particularly useful, with a high resolving power, but often suffer from the disadvantage of being slow and laborious. Recently, *Agilent Technologies* have introduced the Agilent 2100 Bioanalyzer instrument, whereby, compounds can be separated electrophoretically on a single use disposable chip. This type of biochemical analysis, referred to as „lab-on-a-chip" technology, can be used for the separation and analysis of DNA, RNA, cells or proteins.

An Agilent 2100 Bioanalyzer and a Protein LabChip® were evaluated for measuring proteolysis in water-soluble extracts of Cheddar cheese ripened for 3, 6, 9, 12, 15 and 18 months. The „lab-on-a-chip" technology was successfully used in this application, with clear correlations observed between the length of ripening time and the molecular weight of the breakdown products. Compared with slab gel electrophoresis, the „lab-on-a-chip" technology was fast, accurate and reproducible, with the added advantage of a significant reduction in manual labor.

Keywords: proteolysis, cheese, analysis, lab-on-a-chip

P061 COMPARISON OF WATER SOLUBLE PEPTIDE PROFILES FROM CHEDDAR CHEESE MANUFACTURED WITH DIFFERENT *LACTOBACILLUS* ADJUNCT SPECIES

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The objective was to investigate whether increased proteolysis in Cheddar cheese was achievable through addition of selected *Lactobacilli*. Specifically whether hydrolysis of water soluble peptides could be accelerated and concentrations of free amino acids increased.

Replicate 1 kg Cheddar cheeses were made, with and without adjunct cultures, under controlled laboratory conditions using a standardised milk media. NSLAB populations, amino acid concentrations and water soluble peptide profiles were monitored at 1 day, 3 months and 6 months.

The study revealed that *Lactobacillus helveticus* strains readily hydrolysed water soluble peptides and generated higher concentrations of free amino acids. This was not the case for the selected strains from other *Lactobacillus* species, despite equivalent or higher viable populations in the cheese matrix.

Keywords: *Lactobacillus helveticus,* Cheddar cheese, peptides

P062 CONVERSION OF *LACTOCOCCUS LACTIS* CELL ENVELOPE PROTEINASE SPECIFICITY THROUGH PARTIAL ALLELE EXCHANGE

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Bitterness is a common flavor defect in Cheddar cheese that limits consumer acceptance, and specificity of the *Lactococcus lactis* extracellular proteinase (lactocepin) is a key factor in the contribution of starter bacteria to bitterness in cheese. In previous work, we showed *L. lactis* starter cells with group h lactocepin have a high propensity for bitterness, and that this effect was especially pronounced in the early months of ripening where bitterness is a more significant economic concern. Because lactocepin specificity is the primary determinant in whether or not a starter culture produces bitter peptides, the propensity of some industrial strains for bitterness would be attenuated by lactocepin exchange. In this study, we determined the complete nucleotide sequence of *L. lactis prtP* genes encoding group h (bitter) and group b (nonbitter) lactocepins, then utilized a two-step gene replacement strategy to convert substrate-binding determinants in the group h gene of two industrial strains into the corresponding group b sequence. Selection and PCR characterization confirmed that a 2.9-kb fragment of the group h *prtP* regions encoding substrate binding sites had been replaced with the corresponding group b sequence in isogenic derivatives of both strains, and that the cell envelope proteinase in these mutants remained functional. Further characterization of these strains showed no change in acidification activity for one strain, but a slightly slowed rate in the second. Finally, in vitro analysis of lactocepin specificity toward α_{s1}-casein (f 1–23) by reversed-phase high-pressure liquid chromatography demonstrated the lactocepin specificity of both isogenic mutants had been altered to reflect that of group b enzyme.

Keywords: *Lactococcus lactis*, starter culture, lactocepin, bitterness

P063 HOW VOLATILE COMPOUND PROFILES ARE MODIFIED BY INDIGENOUS MILK MICROFLORA IN HARD COOKED CHEESES?

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Indigenous milk microflora is generally considered to contribute largely to the diversity observed in the sensory characteristics both within each cheese (richness) and within a cheese variety (variability among cheeses). Modification of the volatile profiles is probably one of the major ways for generating this diversity. But studies are needed to investigate properly the impact of different levels and compositions of indigenous milk microflora on the richness and variability of volatile profiles, and to link the volatile profiles with the composition and the dynamics of cheese microflora.

An experimentation was designed in which only the population density (2 levels: undetectable, 10^4 cfu ml^{-1}) or the strain composition of indigenous milk microflora (3 levels) varied in the manufacture of twelve hard cooked cheeses. Volatile compounds were analysed by GC-MS after 2.5, 4 and 6 months of ripening.

The three indigenous milk microflora differed totally at strain level, and partially at species level for the two major groups of microorganisms which grow later in cheese. The dynamics of cheese microflora and sensory characteristics differentiated the four types of cheese.

In all cheeses, some volatile compounds accumulated or disappeared rather before 2.5 months, some others all over the ripening, and the others rather after 2.5 months of ripening. Cheeses with and without an indigenous milk microflora exhibited the same volatile molecules, but their balance and/or dynamics were very different until 6 months of ripening. The overall balance of volatile molecules observed with indigenous milk microflora was partially and specifically affected by each microflora composition. Correlation between the profiles of volatile molecules and cheese microflora (composition, dynamics) allowed to investigate the dynamics of cheese ecosystem and the possible sources of diversity of the characteristics of mature cheeses.

Keywords: volatile molecules, hard cooked cheese, indigenous milk microflora, diversity

P064 HIGH THROUGHPUT SCREENING ON FLAVOUR FORMING CAPACITIES TO MAP BIODIVERSITY AMONG LACTIC ACID BACTERIA

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Fermented food products constitute a major part of our daily diet. Fermentation is not only applied as a means of food preservation but special attention is paid to additional benefits such as production of new flavours or increased nutritional value. In recent years, our understanding of the physiological characteristics determining the performance of starter cultures has increased significantly. This is exemplified by the elucidation of the key enzymes and pathways involved in flavour formation in various dairy products. Many key-flavours are derived from proteins by consecutive action of proteolytic/peptidolytic enzymes and amino acid converting enzymes, which are produced by starter organisms.

It is important to realise that various (natural) strains show a large diversity in key enzyme activities. Consequently, high-performing starter cultures can be developed by careful selection and combination of strains with desired activities. This illustrates the need for fast, robust and accurate methods for the determination of key enzymes or flavour metabolites produced by large numbers of diverse microorganisms present in culture collections.

Two examples will be presented of a fully-automated screening method. The first example describes the activity measurement of peptidases in *Lactococcus lactis* strains. The second example describes the screening of these strains for the production of a specific desired key flavour compound. The results show that strains vary highly in both activity levels and regulation of enzyme expression. Moreover, activities were significantly different for the two subspecies (e.g. *lactis* and *cremoris*) and for strains isolated from different sources (e.g. industrial dairy fermentations and non-dairy origin).

Keywords: high throughput screening, biodiversity, flavour, starter cultures

P065 ESTERASE ESTA OF *LACTOCOCCUS LACTIS* MG1363 IS INVOLVED IN THE DEGRADATION OF THIO-ESTERS AND ETHYL ESTERS IMPORTANT FOR CHEESE FLAVOUR

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The role of the sole esterase EstA of *L. lactis* MG1363 in relation to cheese flavour formation is being investigated. By using an *estA* deletion mutant and an *estA* overexpressing mutant of *L. lactis*, we demonstrate that *estA* is involved in the degradation of the potential cheese flavour thio-esters S-methylethanoate, S-methylpropionate and S-methylbutanoate. The ability to degrade these thio-esters increased with increasing acyl-chain length. By using purified EstA the K_{cat} and K_m values were determined for various thio-esters. In addition, the activity of EstA with various food-relevant ethyl esters was demonstrated and also here the hydrolysis increased with increasing acyl chain length (ethylpropionate to ethylhexanoate). The enzyme activity towards thio-esters and *p*-NP esters was reduced more than 600-fold and 800-fold, respectively, at cheese-like conditions at pH 5.5 and 5 % NaCl compared to the activity in 50 mM sodium phosphate buffer at pH 7.0. Upon incubation of the *estA* deletion and overproducing mutants in milk, it was shown that overproduction of EstA resulted in a strong increase in degradation of thio-esters and ethyl esters. Interestingly, the degradation of thio-esters and ethyl esters by EstA was also observed at conditions resembling the cheese matrix, i.e. a cheese paste model system, indicating that EstA is capable to degrade thio-esters and ethyl esters at cheese-like conditions.

Keywords: cheese flavour formation, thio-esters, ethyl esters, esterase

P066 INFLUENCE OF LACTOBACILLUS AS ADJUNCT CULTURE ON THE FREE AMINO ACIDS AND VOLATILE COMPOUNDS OF EWE'S MILK CHEESE

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The addition of selected lactobacilli to cheese milk with the objetive to accelerate cheese ripening or improve cheese quality resulted in increased levels of free amino acids in cheese, accompanied by increased flavour intensity.

The objective of this work was to investigate the effects of *Lactobacillus paracasei /Lb. plantarum* as adjunct culture on the concentrations of free amino acids and volatile compounds in ewe's milk cheese.

Analysis of free amino acids (FAA) and volatile components in a cheese made from pasteurised milk with commercial starter (*Lactococcus lactis*) (P) and in a similar cheese but adding an adjunct culture (FC) was carried out. Two replicate manufacturing runs were made, yielding duplicates of each batch. The adjunct culture was composed by *Lactobacillus paracasei* + *Lactobacillus plantarum* in a proportion of 1:1. The possible relationships between amino acids and volatile components were studied.

The total concentration of FAA increased 11–12 times from 1 to 240 days of ripening at 12 °C. The main amino acids, which accounted for about 70% of the total FAA were Leu (18%), Glu (16%), Lys (14%) and Phe, Val and Ile (about 7% each). At the end of the ripening process, there were significant differences of Leu, Ile, Gaba, Phe, Pser, Ser, Gln, Ala and Orn. Higher concentrations of those, were found in the batch FC, apart from Ser and PSer, that were found in higher concentrations in the batch P.

Seventy three volatile components were identified, comprising: seventeen hydrocarbons and alcohols, two sulphur-containing compounds, eleven aldehydes, twelve ketones, six acids and eight esters.

The batch FC, at 240 days of ripening, showed significantly higher levels of benzene, toluene, ethanol, 2-methylpropanol, 3-methylbutanol, 2-methylpropanal, 3-methylbutanal, propan-2-one, butan-2-one, pentan-2-one, diacetyl and acetoin, acetic acid, isobutyric acid, butanoic acid, isovaleric acid, methanoic acid ethyl ester, acetic acid ethyl ester, methanoic acid butyl ester and butanoic acid ethyl ester.

Correlations were found between different amino acids and the compounds resulting from their metabolism.

Keywords: ewe milk cheese, adjunct culture

P067 GENERATION OF VOLATILE COMPOUNDS BY MICROORGANISMS FROM A PORTUGUESE TRADITIONAL CHEESE

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The contribution of selected microorganisms, previously isolated from ripened Serra da Estrela cheese to the formation of volatile compounds was evaluated in this research effort. Curdled ewe's milk inoculated with the selected isolates was used as model system in attempts to follow amino acid breakdown and the formation of volatile compounds at 10°C with sampling at 0, 7, 14, 28 and 42 d. Pilot-scale experimental cheeses were manufactured using also those isolates, and allowed to ripen at 10 °C with sampling at 0, 7, 14, 28, 42 and 63 d.

In the model system, phenylalanine (Phe), glutamic acid (Glu) and proline (Pro) were the free amino acids found in highest amounts. In cheese, leucine (Leu), valine (Val) and Phe were the major free amino acids found; the amount of Leu was 2–3-fold higher than Val. The levels of almost all amino acids increased throughout ripening, hence suggesting their gradual release into the cheese matrix via proteolytic breakdown. Volatile free acids were quantified, since they are known to play a key role in the aroma profile of this cheese. In both matrices (model system and cheese), these increased throughout the ripening process. In the model systems, iso-valeric acid (iC5) was always the most abundant volatile (2–3-fold higher than the other volatile free acids). However in cheese, the most abundant were butyric and caprylic acid. These are known to be strongly flavourful, even at minimal levels; therefore, their presence in Serra da Estrela cheese has been claimed to be a positive attribute. Addition of lactic acid bacteria as starter cultures (either individually or as a mixture) in the manufacture of Serra da Estrela cheese will likely result in safer cheeses (with lower microbial loads of potentially pathogenic bacteria), while preserving the unique flavour attributes.

Keywords: Free volatile acids, free amino acids, lactic acid bacteria, Serra da Estrela cheese

P068 MONITORING VOLATILE COMPOUNDS WITH SPME-GAS CHROMATOGRAPHY DURING MOULD CHEESE RIPENING

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Flavour is one of the most important quality criteria that influences the selection and consumption of food. Volatile compounds potentially involved in cheese flavour are hydrocarbons, alcohols, aldehydes, ketones, esters, fatty acids, lactones as well as sulphur- and nitrogen-containing compounds. Cheese flavour is formed during ripening of cheese and results from the breakdown of milk proteins, fat, lactose and citrate due to enzymes from microorganisms, coagulants and milk. Mould growth has been thought to play a major role in the production of volatile flavour compounds and texture modifications of mould cheeses.

The solid-phase microextraction (SPME) procedure coupled to gas chromatography was used to study the evolution of volatile compounds of Niva cheese during ripening. Niva is a mould ripened cheese (50 % fat in dry matter) manufactured (under the usual conditions) according to the traditional procedures in a dairy in Česky Krumlov. SPME is a new sample preparation technique based on the partition of the analyte between the extraction phase on the outside of a small fused-silica fibre and the matrix. The last few years, SPME has been applied to various biological samples such as, e.g., urine, plasma and hair. It was also used in food analysis including a study of vegetables and fruits, juices and other soft drinks, alcohol beverages, cheese and dairy products. SPME-headspace is a very interesting sampling possibility for solid and liquid samples in food flavour analysis.

The aim of our work was to develop a simple, rapid, cheap and reliable method for the analysis of the volatile flavour compounds of cheese. Changes throughout mould cheeses ripening were monitored using this method. Our results would provide important information contributing to knowledge of the biochemical processes involved in the ripening.

Keywords: mould cheese, flavour, SPME, gas chromatography

P069 FREE FATTY ACID EVOLUTION IN CAMEMBERT CHEESE DURING RIPENING

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Phenomena generating free fatty acids and their derivatives in cheese are a major aspect of ripening. Experimental Camembert-type cheeses were prepared in duplicate from pasteurised milk. The ripening flora was *Kluyveromyces lactis* (44_s, GMPA collection), *Geotrichum candidum* (D, Degussa), *Penicillium camemberti* (R, Degussa) and *Brevibacterium linens* (ATCC9175) under aseptic conditions. Two ripening replicates, performed under 12 °C, 95 % of relative humidity and atmosphere controlled conditions, showed similar ripening characteristics during 45 days. For each cheese, the FFA concentrations varied with the part considered (rind or core). The evolutions of FFA concentrations were studied during ripening. These components were extracted with diethyl ether and methylated with TMAH, separated and quantified by direct gas chromatography and identified by mass spectrometry. The concentrations of valeric, heptanoic, undecanoic, pentadecanoic and heptadecanoic acids, which were negligible whatever the time, were used as internal standards. The degree of lipolysis was shown by the increase in concentration of FFAs during ripening. During the ripening the FFA production was best correlated with the starter strains, especially with *G. candidum* and *P. camemberti*. The evolutions of hexanoic and capric acids were similar and were associated with the concentrations of *K. lactis* and *G. candidum*. The ones of octanoic and lauric acids were also similar and were strongly related to *G. candidum* and *P. camemberti*. For these four FFAs the concentration in the rind, versus time, presented the same evolution than that observed in core. Myristic, palmitic and stearic acids were the most abundant. The concentrations in the rind were associated with *P. camemberti* spores and *G. candidum* viable cells. FFA accumulations showed a maximum on day 15 (packaging). After day 15, their concentrations remained stable. This could be explained by the â-oxidation metabolism due to *G. candidum* and *P. camemberti*.

Keywords: Free fatty acid, surface microflora, Camembert

P070 BIOSYNTHESIS OF DIMETHYL SULFIDE BY CHEESE-RIPENING MICROORGANISMS: USE OF POSSIBLE PRECURSORS

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Numerous cheese-ripening microorganisms, including yeasts and bacteria, are able to produce volatile sulfur compounds such as thiols, methyl sulfides, thioethers or methylthioesters. Their low odor thresholds make an important contribution to the odor and aroma of cheeses and may interact with the organoleptic properties of cheeses. Among such compounds, dimethyl sulfide (DMS) has been less studied, although its production by several cheese-ripening yeasts and bacteria has already been reported (Bonnarme et al. 2001).

Twelve yeasts and sixteen bacteria were cultivated in the presence of three common DMS precursors: *S*-methylmethionine (SMM), dimethylsulfoxide (DMSO) and dimethylsulfoniopropionate (DMSP). The production of DMS was investigated for each strain and precursor. The effect of temperature and pH of incubation was also studied on the most efficient DMS producers. For some microorganisms, DMS production kinetics were also reported.

Seven yeasts and thirteen bacteria were able to produce DMS from SMM while nine yeasts and twelve bacteria were able to reduce DMSO to DMS. In contrast, DMSP appeared to be a very poor DMS precursor both for yeasts and bacteria species contrarily to what was already described for macroalga or bacteria isolated from marine ecosystems (De Souza and Yoch, 1995).

On the basis on these first results, best DMS producers, namely *Saccharomyces cerevisiae* L36, *Yarrowia lipolytica* L91 and the bacterium *Corynebacterium* sp C74, were selected for further investigations. DMSO and SMM were used as DMS precursors. The effect of pH and temperature on DMS production was evaluated. Kinetics studies show that DMSO reduction to DMS by yeast *S. cerevisiae* L36 is well correlated with its growth. SMM was most efficiently cleaved to DMS by *Corynebacterium sp* C74. The involvement of enzymatic activities possibly involved in DMS production is discussed.

This work gives evidence that some cheese-ripening microorganisms are able to produce consistant amounts of DMS from at least two precursors, DMSO and SMM. Although these two common DMS precursors were evidenced in other food stuffs such as beer, their occurrence in cheeses remains questionable. However studies have shown that SMM was an excellent DMS precursor for the yeasts *G. candidum* and *Y. lipolytica* (Spinnler et al. 2001). A next step could therefore be, i) to study the conversion of L-methionine – a common sulfur amino acid found in cheese – to DMS, ii) to investigate the conversion of L-methionine to SMM, and its subsequent degradation to DMS.

Keywords: cheese-ripening microorganisms, dimethyl sulphide, *S*-methylmethionine, dimethylsulfoniopropionate, dimethylsulfoxide

P071 IMPACT OF CITRATE METABOLISM ON AMINO ACID CATABOLISM BY *L. LACTIS DIACETYLACTIS*

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Amino acid catabolism is a major pathway for aroma formation by lactic acid bacteria (LAB). However, amino acid catabolism by LAB is often limited by the low production of α-ketoacids required as amino group acceptors in amino acid transamination, the first step of amino acid catabolism. Theoretically, catabolism of citrate by citrate utilizing LAB can generate 2 α-ketoacids (pyruvate and α-ketoglutarate (α-kg) in the presence of glutamate) via the action of citrate permease, citrate lyase and oxaloacetate decarboxylase or aspartate aminotransferase.

The objective of the study was to investigate the impact of citrate catabolism by *L. lactis diacetylactis* (citrate utilizing LAB) on the formation of pyruvate and α-kg and their further utilisation for amino acid catabolism.

To do that, we used 2 strains of *L. lactis diacetylactis* and 1 strain of *L. lactis lactis*, each containing or not a plasmid carrying the gene encoding citrate permease that allows citrate uptake inside the cells. Resting cells of these strains were incubated in a reaction medium containing citrate and glutamate and with or without phenylalanine or leucine. Citrate utilisation, pyruvate and α-kg production and Phe and Leu catabolism were monitored during a 10 h incubation at 37 °C.

Only the strains possessing citrate permease and citrate lyase (*L. lactis diacetylactis*, citrate permease positive) were capable of utilizing the citrate present in the reaction medium. They produced significant amount of pyruvate and α-kg and catabolised phenylalanine and leucine when they were present in the medium.

These results show that citrate catabolism stimulates amino acid catabolism *via* the production of α-kg and pyruvate. However the respective impact of pyruvate and α-kg on amino acid catabolism remains to be evaluated.

Keywords: amino acid catabolism, citrate catabolism, *L. lactis diacetylactis*, aroma formation

P072 5'-RIBONUCLEOTIDE FLAVOR ENHANCER CONTENT AND ENDORIBONUCLEASE ACTIVITY IN CHEDDAR CHEESES

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Nucleic acids and nucleases are abundant in cheese as part of microbial cells. The 5'-ribonucleotide degradation products of RNA are of particular interest due to their known function as flavour enhancers. Guanosine 5'-monophosphate (5'-GMP) and inosine 5'-monophosphate (5'-IMP) are used as food additives for their flavour potentiating properties. The natural occurrence of 5'-ribonucleotides and ribonucleases in cheese has not been well characterized. The objectives were to develop a method for extracting and quantifying 5'-ribonucleotides in cheese and to determine the 5'-GMP and 5'-IMP concentrations and endoribonuclease activity in commercial Cheddar cheeses. To analyse 5'-ribonucleotides in cheese, a water soluble fraction was prepared in RNase-free water. This fraction was extracted with perchloric acid (PCA). The PCA-soluble fraction was subjected to high performance liquid chromatography through a C-18 reversed phase column with potassium phosphate (pH 6.0) as the mobile phase. Nucleotides were detected by absorption at 260 nm. Sixteen commercial Cheddar cheeses were evaluated. The concentrations of 5'-GMP and 5'-IMP ranged from 0 to 7 µg/g cheese and from 0 to 20 µg/g cheese, respectively. Eleven cheeses contained both compounds, whereas neither compound was detected in three cheeses. In two cheeses, only 5'-GMP was detected. Water-soluble fractions from the same cheeses were evaluated for endoribonuclease activity using a fluorescent RNA probe with bovine pancreatic RNase A as the standard. Endoribonuclease activities ranged from 0.26 to 2.3 units/g cheese. There was no apparent relationship between endoribonuclease activity and 5'-GMP and 5'-IMP contents at the sampling time. Among commercial Cheddar cheeses, much variation was observed in endoribonuclease activity and ribonucleotide flavour enhancer content. A fundamental understanding of the sources and importance of all potential flavour compounds is essential for controlling cheese flavour. Establishing the presence of and variability in 5'-GMP and 5'-IMP concentrations in Cheddar cheeses will contribute to further understanding of cheese flavour.

Keywords: ribonucleotide, Cheddar, ribonuclease, flavour

P073 FORMATION OF FREE FATTY ACIDS, LACTONES AND ESTERS DURING GOUDA CHEESE RIPENING

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Flavour is an important cheese characteristic, which is notably formed during the ripening phase from protein and lactose. However, besides compounds derived from protein and lactose, a balanced cheese-flavour also relies on aroma compounds derived from milk fat. These include fatty acids, lactones, esters and ketones. It is unknown whether their formation is due to enzymatic activity (lactic acid bacteria, non-starter lactic acid bacteria, or other enzymes) or due to chemical reactions. Therefore, it is valuable to know more about their formation during cheese ripening. This poster presents the results from Gouda cheese ripening studies, using a newly developed method for the analysis of these compounds.

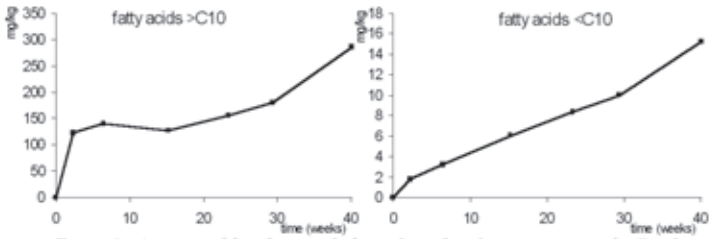

Figure 1. Amounts of free fatty acids formed (mg/kg) during ripening of a Gouda cheese produced from pasteurised milk

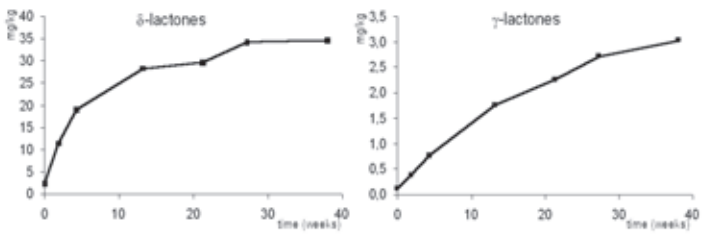

Figure 2. Amounts of δ-and γ-lactones (mg/kg) produced during ripening of a Gouda cheese produced from pasteurised milk

Figure 1 shows that long chain free fatty acids were produced in the first 2, and after 20 weeks of ripening, while short chain free fatty acids were produced in a rather linear manner for at least 40 weeks of ripening time. This suggested that two different enzymes were involved in the production of free fatty acids in Gouda cheese, but more information about the nature of these enzymes cannot be deducted from this data.

Figure 2 shows that the formation δ-lactones was very fast in the beginning, but slowed quickly and seemed to come to a standstill after about 30 weeks of ripening. This decrease in the formation rate of γ-lactones was lower, and some production still was observed after 30 weeks of ripening. Lactones are thought to originate from hydroxy acids, but their formation is different from other fatty acids. They might also be formed from free fatty acids.

Esters produced in Gouda cheese were mainly long chain ethyl esters, which were produced linearly throughout ripening. Cheese from a raw milk cheese showed 10-fold more ethyl esters than in cheese from pasteurised milk, which suggested some influence of non-starter lactic acid bacteria.

Keywords: cheese ripening, free fatty acids, lactones, esters

P074 USE OF SOLID PHASE MICRO EXTRACTION (SPME) TO STUDY THE VOLATILE COMPOUNDS EVOLUTION IN SARDINIAN OVINE BLUE CHEESE DURING RIPENING

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Blue cheese is a general classification of cow's, goat's and ewe's milk cheeses containing a blue or blue-green mould. The most known are: Gorgonzola (Italy), Danablu (Denmark) made with cow's milk, and Roquefort (France) produced with sheep milk. In Sardinia, a small production of ovine blue cheese is made at industrial level.

The dominating flavour in these cheeses is due particularly to the presence of free fatty acids and to their degradation products, such as methyl-ketones. Various analytical and instrumental methods have been developed to determine these compounds in the cheese volatile fraction. Most of these methods use high solvent volumes or special apparatus and are time consuming.

SPME is a relatively new analytical technique that can overcome these difficulties. Therefore a suitable method was developed to study the volatile compounds in Sardinian ovine blue cheese. For this purpose a 50/30 DVB/Carboxen/PDMS fiber allowed us to obtain an equilibrate profile between non-polar and polar volatile compounds. For 24-h and 1-month old cheeses, a sampling time of 20 min and 7 min was selected, respectively. In these conditions both a higher signal intensity for the volatile compounds and a correct ratio C4:0/C6:0 were obtained. The coefficient of variation (CV) for 3 replicates ranged from 1 to 29 %.

About 46 compounds were identified in the cheese samples analysed. In the ripened cheeses 68 % of volatile compounds were metyl-ketones. Among these, the most represented were 2-pentanone, 2-heptanone and 2-nonanone. Free fatty acids were 31%, where butyric and caproic acids were the most represented.

Keywords: Ovine blue cheese, SPME, GC-MS, volatile compounds

P075 ROLE OF POTENTIAL HYDROXYACID-DEHYDROGENASES OF *LACTOCOCCUS LACTIS* IN AMINO ACID CATABOLISM

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In lactococci, the first step of the conversion of aromatic and branched-chain amino acids into aroma compounds is a transamination, which transforms the amino acids into α-ketoacids. These α-ketoacids can then be transformed into diverse compounds with more or less aroma. In cheese, a large part of α-ketoacids is transformed to hydroxyacids, which are not aroma compounds. Since this pathway of α-ketoacid conversion competes with the pathways leading to aroma compounds, its interruption could allow to intensify aroma formation in cheese (Yvon and Rijnen, 2001). Hydroxyacid-dehydrogenases active towards α-ketoacids derived from amino acids have been isolated from several lactobacilli and have been named hydroxyisocaproate-dehydrogenases (Hic-DH). In *L. lactis* IL 1403, five genes, in addition to the gene encoding the lactate dehydrogenase (LDH), share high homology with these Hic-DHs (Bolotin et al., 2001).

To identify the genes encoding the enzymes responsible for the reduction of aromatic and branched-chain α-ketoacids in *L. lactis*, the five genes homologous to the Hic-DHs and that encoding LDH were inactivated in *L. lactis* TIL46. We studied then the effect of the inactivations on the reduction of different α-ketoacids. None of the single inactivations of the Hic-DH homologous genes reduced the activity of cell extract towards the studied substrates. In contrast, inactivation of the LDH gene highly reduced the activity towards pyruvate and phenylpyruvate, an aromatic α-ketoacid, while it did not modify the activity towards α-ketoisocaproate, a branched-chain

α-ketoacid. These results indicate that LDH is mainly responsible for the reduction of aromatic α-ketoacids but none of the inactivated gene is responsible alone for the reduction of branched-chain α-ketoacids. Either compensatory regulations take place when one gene is inactivated or another gene is involved in this pathway. The later question will be addressed by screening a mutant library for α-keto-isocaproate reduction to identify the gene responsible for this activity.

Keywords: *Lactococcus lactis*, amino acid catabolism, α-ketoacid reduction

P076 EXPRESSION OF THE GENE ENCODING THE ESTERASE IN *LACTOCOCCUS LACTIS*

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Lactococcus lactis NCDO 763 produces an esterase, EstA, capable of either hydrolysing or synthesising aromatic esters from alcohols and short-chain fatty acids, depending on the environmental conditions (1). The gene encoding EstA (*estA*) seems to be localised in a 11kb operon containing also genes encoding proteins involved in regulation, transport and competence processes. However the precise role of EstA in bacteria remains unknown.

To specify the function of EstA in *L. lactis* NCDO 763, we monitored the esterase activity as well as the gene expression during the growth in different culture media. Esterase activity was measured with butyryl-pnitrophenyl and gene expression was analysed by quantitative RT-PCR.

Esterase activity and *estA* gene expression were optimal in the middle of exponential growth phase in M17 medium and at the end of the exponential growth phase in skimmed milk. Thereafter, i.e. during stationary phase, *estA* expression was totally repressed. However, the optimal levels of activity and gene expression were similar in both media. The addition of tributyrine to the skimmed milk only slightly reduced the optimal activity.

We concluded that the expression of *estA* is regulated during growth at the transcriptional level and that this regulation occurs at different growth states, depending on the growth medium. Neither tributyrine nor fat seem to be effectors of the regulation suggesting that EstA has another function than fat hydrolysis. Therefore, the factors involved in the regulation remain to be identified.

Keywords: *Lactococcus lactis*, esterase, gene expression

P077 DIACETYL AND ACETOIN FORMATION BY *LACTOCOCCUS LACTIS* VIA ASPARTATE CATABOLISM

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Diacetyl and, to a lesser extent, acetoin are major aroma compounds in fermented dairy products. In Cheddar and Gouda type cheeses, they are responsible for a sweet and creamy note that is very appreciated by consumers. Generally these compounds are produced from citrate by certain lactic acid bacteria such as *L. lactis diacetylactis*. Citrate catabolism generates a pyruvate excess, which is partly transformed to diacetyl and/or acetoin. However, it appears that these compounds could also be formed from amino acid catabolism. Indeed, in Cheddar cheese, diacetyl and acetoin formation was stimulated by the addition of α-ketoglutarate, which stimulates amino acid catabolisms involving a transamination step (1,2).

The objective of the study was to evaluate the ability of *L. lactis* to produce diacetyl and acetoin from aspartate catabolism, since an aspartate catabolism pathway is initiated by a transamination step and generates pyruvate.

Aspartate catabolism by *L. lactis* was studied by incubating resting cells in different reaction media containing ^{13}C aspartate and containing or not α-ketoglutarate. Diacetyl and acetoin were then analysed by gas chromatography and mass spectrometry and other metabolites were analysed by HPLC.

Acetoin and diacetyl were only produced in reaction media containing both aspartate and α-ketoglutarate. Mass spectra of formed acetoin and diacetyl revealed the incorporation of 2 or 4 ^{13}C atoms, demonstrating that aspartate was the precursor. Other metabolites formed from aspartate were also identified. The main ones were pyruvate, lactate and acetate. The amounts of each produced metabolite depended on the pH of reaction medium. Indeed, the production of different metabolites results of the activity of the different enzymes involved in pyruvate formation (aspartate aminotransferase, oxaloacetate decarboxylase) and its catabolism (acetolactate synthase, lactate dehydrogenase, pyruvate dehydrogenase, …).

Other studies are in progress to verify if catabolism of other amino acids can also generate these two aroma compounds.

Keywords: diacetyl, *L. lactis*, aspartate catabolism, aroma formation

P078 EXTRACELLULAR REDOX POTENTIAL AFFECTS THE AMINO ACID CATABOLISM BY *LACTOCOCCUS LACTIS*

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Amino acid catabolism by lactic acid bacteria (LAB) plays a major role in formation of aroma compounds in hard and semi-hard cheeses. This process is influenced by several environmental factors such as pH, salt concentration, water activity and temperature. Although many reactions of oxido-reduction type are involved in amino acid catabolism, so far nothing is known about the impact of redox potential on the nature of metabolites produced in cheese by LAB.

In this study, we investigated in vitro the impact of extracellular redox potential on catabolism of phenylalanine, methionine, and leucine by two strains of *Lactococcus lactis*. The study was performed in a buffer solution at pH 5.5, in the presence of α-ketoglutarate and with radiolabelled amino acids as tracers. The redox potential (Eh) was around +200mV. The reducing conditions (Eh = -200 mV) were obtained by addition of dithiothreitol, while very oxidative conditions (Eh = + 400mV) were obtained by addition of potassium ferricyanide.

Redox potential considerably affected the amino acid catabolism by *Lactococcus lactis*. However in all cases the first step of amino acid catabolism was a transamination that produces α-ketoacids. Under reducing conditions both strains produced mainly hydroxyacids and carboxylic acids from phenylalanine, leucine and methionine. Probably, the reduction of α-ketoacids to hydroxyacids (which required NADH) regenerated NAD^+ that was used subsequently by α-ketoacid dehydrogenase to convert the α-ketoacids into carboxylic acids. Generally, oxidative conditions rather favoured production of the aldehydes resulting from α-ketoacid decarboxylation as well as the metabolites formed by chemical oxidation of α-keto acids, such as methanethiol and dimethyldisulfide from methionine and benzaldehyde from phenylalanine.

We concluded that redox potential all along the cheese making and ripening could have a considerable impact on the nature of metabolites produced by LAB and consequently on cheese aroma.

Keywords: redox potential, *Lactococcus lactis*, amino acid catabolism

P079 THE USE OF A CARBOXYPEPTIDASE FOR ACCELERATED CHEESE RIPENING

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Acceleration of cheese ripening requires the rapid development of flavours and flavour precursors. An important group of flavour precursors are the amino acids, which are liberated by proteases in the cheese and are subsequently converted to flavour compounds by the microbial cheese flora. Since the availability of amino acids, and subsequent conversion, is known to be a bottle neck in cheese flavour development, processes to facilitate amino acid generation are expected to accelerate cheese ripening. Of particular importance is the liberation of amino acids that are important for cheese flavour development like phe, leu, met and val. In this poster we will present results we obtained in accelerating cheese ripening with a broad spectrum carboxypeptidase. We demonstrate that the carboxypeptidase is able to liberate almost any amino acid from peptide-substrates, but that it is particularly active on phe, leu, met and val. The carboxypeptidase was used in the preparation of Cheddar-type and Gouda-type cheeses. Taste panel assessment of the cheeses after 3 and 6 weeks of ripening in the case of the Cheddar-type cheeses and 6 weeks and 3 months of ripening in the case of the Gouda-type cheeses with and without the carboxypeptidase, revealed a clear enhancement of cheese ripening in both types of cheese when the carboxypeptidase was added. The pH profile of the carboxypeptidase was determined, showing little to no activity at pH > 6 and increasing activity at lower pH values with maximum activity between pH 3–4. This pH profile is better compatible with cheese application compared to those of aminopeptidases, which usually have a pH optimum at pH 6.5 or higher.

Keywords: cheese ripening, acceleration, carboxypeptidase

P080 PRODUCTION OF BRANCHED-CHAIN AROMA COMPOUNDS BY THREE CHEESE-RELATED *LACTOBACILLUS* SPECIES

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Several branched-chain flavour compounds derived from the catabolism of branched-chain amino acids are produced during the ripening of Emmental cheese, but the respective contribution of each strain or species of the cheese microflora is unknown.

In this study we compared the ability of three *Lactobacillus* species to produce branched-chain volatile compounds in vitro. Two thermophilic starters, *Lb. helveticus* (4 strains) and *Lb. delbrueckii* spp. l*actis* (3 strains), and one non-starter species, *Lb. paracasei* (3 strains) were used. Resting cells were incubated for 160 h at 24 °C in phosphate buffer, 60 mM, pH 5.6, à 24 °C, in the presence of either a mixture of branched-chain amino acids (leucine, isoleucine and valine, 3 mM each), or α-ketoglutaric acid alone (K, 10 mM) or a mixture of branched-chain amino acids and of α-ketoglutaric acid (AK). Alfa-ketoglutaric acid is an amino group acceptor required for amino acid transamination. A control without cells was also included. Glutamate, produced by transamination was measured enzymatically and volatile compounds were analysed by gas chromatography – mass spectromethy.

The production of glutamate and volatile compounds was not detected in the controls. All tested strains showed transamination of branched-chain amino acids, with a net production (AK – K) ranging from 1 to 4 mM. Volatile compounds were produced only when amino acids were added (AK) by all the strains tested, and their concentrations were highly strain-dependant. Branched-chain acid concentrations ranged from < 0.20 mM (6 strains) to 2.56 mM, branched-chain alcohol concentrations from < 0.005 mM (8 strains) to 0.93 mM, and branched-chain aldehyde concentrations from < 0.005 mM (8 strains) to 0.42 mM. The proportion of compounds derived from leucine, isoleucine and valine, respectively, were also found to be strain-dependant. Greater differences in the production of volatile compounds were observed intra species than inter species under the stated experimental conditions. The ability of *Lactobacilus* strains to produce branched-chain volatile compounds will now be determined in cheese.

Keywords: volatile compounds, branched-chain amino acid, catabolism, *Lactobacillus*

P081 A SIMPLE SCREENING METHOD FOR THE PRODUCTION OF ISOVALERIC ACID BY *PROPIONIBACTERIUM FREUDENREICHII* IN SWISS TYPE CHEESE

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Isovaleric acid (3-methylbutyric acid) and 2-methylbutyric acid are cheese flavour compounds that are produced in Swiss-type cheeses mainly by propionibacteria (PAB), with amounts produced being strain-dependant. To evaluate the possibility of screening this property in vitro, the production of both acids (referred to as methylbutyric acids) was compared for eight *Propionibacterium freudenreichii* strains both in mini-Swiss cheeses and in liquid cultures (salted-yeast extract-peptone-lactate medium, pH 5.4, incubated at 24 °C then at 6 °C). Similar time courses of methylbutyric acid production were observed in cheeses and in liquid cultures. PAB produced methylbutyric acids during propionic fermentation at 24°C and during further storage at 6 °C for 2 months. PAB strains significantly ($P < 0.05$) influenced the amounts produced, and they were similarly classified in cheeses and in liquid cultures. This simple screening method has potential for selection of strains producing high or low amounts of isovaleric acid for cheese manufacture.

Keywords: *Propionibacterium*, isovaleric acid, Swiss cheese, screening method, flavour compound

P082 PRODUCTION OF VOLATILE COMPOUNDS BY *PROPIONIBACTERIUM FREUDENREICHII* IN SWISS TYPE CHEESE

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Cheese flavour results from the complex mixture of volatile compounds originating mainly from the enzymatic degradation of curd components by cheese microflora during cheese ripening. Directing cheese flavour development requires knowledge of the respective contribution of each of the cheese microflora.

Volatile (flavour) compounds produced by *Propionibacterium freudenreichii,* constituting the main secondary flora of Swiss cheese, were identified by gas chromatography–mass spectrometry in mini Swiss cheeses, and also in pure cultures of propionibacteria grown in cheese aqueous phase (juice). Selected compounds were quantified using calibration curves that were based on the addition of standards to juice or to cheese homogenate samples.

Propionibacteria significantly influenced the quantities detected of 57 out of the 69 volatiles identified in juice. Short-chain carboxylic acids (acetic, propionic, butanoic, hexanoic and isovaleric acids), esters (mainly esters of acetic and propionic acids), and some ketones and alcohols were more abundant in the presence of propionibacteria, whereas most aldehydes were less abundant. Many of these volatiles were branched-chain compounds. The presence of *P. freudenreichii* in mini Swiss cheese generated similar changes in the volatile profile as in juice, although changes were less pronounced. Carboxylic acids and esters are the most probable cheese flavour-active compounds produced by propionibacteria.

Keywords: *Propionibacterium*, volatile compounds, Swiss cheese, GC-MS

P083 THE USE OF MESOPHILIC LACTOBACILLI WITH GLUTAMATE DEHYDROGENASE ACTIVITY AS STARTER ADJUNCTS IN CHEDDAR CHEESE MANUFACTURE

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Glutamate dehydrogenase (GDH) is considered a key enzyme required for cheese flavour generation. The current study investigates the potential use of adjunct mesophilic lactobacilli with GDH activity to influence cheese flavour development. GDH activity was detected in all *Lactobacillus plantarum* strains tested but was usually absent in *Lb. paracasei* strains. Cheddar cheese was manufactured in triplicate using GDH positive *Lb. plantarum* DPC 2109 (G+) and GDH negative *Lb. paracasei* DPC 2022 (G-) strains as adjunct cultures. Four vats of cheese were manufactured in each trial containing either (a) no adjunct (Control), (b) G- adjunct, (c) G+ adjunct and (d) G- plus G+ adjuncts. Adjuncts were added at starter addition at ~10^4cfu/ml milk. After manufacture the chemical composition of all vats was within the required parameters for good quality Cheddar cheese. The addition of the adjuncts had no effect on make times of the cheese. At day 1, lactobacilli were absent from the control vat but were detected in the experimental vats at ~10^6 cfu/g of cheese. By day 14 lactobacilli were detected at ~10^8 cfu/g in the experimental vats and remained fairly constant throughout 6 months ripening. There was a slight decrease in numbers in vat (c) containing DPC 2109 after 3 months ripening but numbers remained above 10^7 cfu/g throughout. Lactobacilli did not exceed 10^3 cfu/g at 6 months in the control vats. Pulsed field gel electrophoresis analysis of 20 lactobacillus isolates, taken from the highest countable dilution of each cheese at 6 months showed that those strains that were added to the vat were present at high numbers. In vat (d) containing both G+ and G- adjuncts the *Lb. paracasei* strain dominated but the *Lb. plantarum* strain was still present. The effect of the adjunct cultures on flavour and on the production of volatile compounds at 6 months will be demonstrated.

Keywords: GDH, mesophilic lactobacilli, flavour

P084 ACIDIFYING AND AROMATIC PROPERTIES OF *ENTEROCOCCUS* STRAINS IN OVINE AND BOVINE MILKS

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Enterococci are lactic acid bacteria present in a wide variety of dairy and other food products. Although their presence in the former has been considered an indicator of poor hygienic conditions, nowadays a number of biotechnological and probiotic useful characteristics are already known. A few compounds resulting from the bacterium metabolism contribute to the aroma of cheese. Knowledge of those components, as well as of the acidifying capacity is very important to the dairy industry, including application toward selection of strains as better starters. The aim of this research effort was to study the role of some enterococci in ovine and bovine milks, in terms of acidifying properties and aroma potential.

A total of seven strains, previously isolated from traditional Portuguese cheeses, were assayed for: *Enterococcus faecium* 28A, *E. durans* 13 and *E. faecalis* 6 (from Serra da Estrela cheese); and *E. faecium* 42, *E. durans* 15, *E. faecalis* 3 and *E. casseliflavus* 40 (from Terrincho cheese). Sterilized ovine and bovine milks were inoculated with 2% (v/v) inoculum and incubated at 37 °C. Microbiological viable counts on M17 agar, pH and titrable acidity were determined in duplicate at 0, 1, 4 and 7 d of incubation. Free fatty acids, recognized as strong odorants in Serra da Estrela and other traditional Portuguese cheeses, were quantified in each sample using SPME – GC/MS.

All strains showed similar growth patterns, either in bovine or ovine milks, and reached levels of 10^8–10^9 cfu/ml by 1 d of incubation (except *E. durans* 13, which exhibited a better growth in bovine than ovine milk). An important decrease in pH was observed in both types of milk by 1 d, for all strains. The highest reduction was observed for *E. casseliflavus* 40 and *E. durans* 15 in bovine milk, and for *E. faecium* 42 and *E. durans* 13 in ovine milk. *Enterococcus faecalis* 6 revealed the poorest acidification power.

Keywords: enterococci, cheese, volatile compounds, acidification

P085 SENSORY AND CHEMICAL PROPERTIES OF WHITE PICKLED CHEESE PRODUCED BY USING KEFIR, YOGHURT AND COMMERCIAL CHEESE CULTURE AS A STARTER

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In this study, sensory and chemical properties of white pickled cheese produced by using kefir, yoghurt and commercial cheese culture as a starter were examined. In white pickled cheese samples produced by using different cultures, acidity, pH, total solids, fat, protein, salt, ash, water soluble nitrogen values were determined, also salt and fat in total solids and ripening degree values were calculated on the 15 day intervals during 120 days of ripening period. Moreover, appearance, structure, odour and taste of the cheese samples were determined.

According to the statistical evaluations carried out, it was determined that the effect of the culture variety on acidity, pH, salt, fat and salt in total solids values in the cheese samples was significant ($p<0.01$); that its effect on ash, appearrance and odour properties was significant ($p<0.05$). Regarding the ripening period, it effected titrable acidity, pH, total solids, fat content, protein content, water soluble nitrogen, ripening degree and scores of texture, odour, taste and over all sensory properties significantly ($p<0.01$) and also it has a significant effect ($p<0.05$) on ash content. The amounts of fat in the total solids in cheese samples was not effected by the culture variety and ripening period. In white cheese samples produced by using kefir culture appearance, structure and odour got the highest points. It was determined that culture variety did not have a statistically effect on cheese taste and it was concluded that kefir can be used as a starter culture in production of white pickled cheese.

Keywords: starter culture, kefir, white pickled cheese, chemical and sensory properties

P086 DIFFERENTIATION OF LACTIC ACID BACTERIA USING AN ELECTRONIC NOSE

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Facultatively heterofermentative *Lactobacillus* were isolated from Gruyère cheese (Swiss raw milk hard cheese) manufactured at four different cheese factories. These cheeses were noted by a panel of tasters. Strains of *Lactobacillus paracasei* and *Lb. rhamnosus* were found in the cheeses. The strains were classified into genetic variants using two methods, REP-PCR with $(GTG)_5$ primer and sequencing of an intergenic region of the chromosome of *Lb. paracasei*. The cheeses were shown to contain different genetic variants, which were found in several cheeses.

Reference strains of lactic acid bacteria, propionibacteria and different genetic variants of *Lb. casei* and *Lb. rhamnosus* from cheeses were selected for analysis with an electronic nose (SMARTNOSE, LDZ, Switzerland). The strains were incubated in a complex growth medium, centrifuged and resuspended in whole UHT milk supplemented with a cocktail of amino acids. The volatile compounds produced were analysed after incubation for ten days at 30 °C. The electronic nose used does not detect compounds individually after chromatographic separation, but it analyses the sum of the volatiles. The spectra of the masses are treated by multivariate analysis. Principal component analysis showed that the bacterial samples differed from the controls (water and uninoculated milk), indicating that volatile compounds were produced by the bacterial cultures. The different bacterial species clustered separately. Moreover, strains of the same genetic variants formed distinguishable groups, some of them completely separated from the others. Our results showed that it is possible to group genetically closely-related micro-organisms on the basis of their production of volatile compounds. Although some methodological improvements are necessary to increase the reproducibility of the results, this method is a powerful technique for screening aroma producers and for differentiating strains.

Keywords: lactic acid bacteria, *Lactobacillus*, electronic nose, screening technique

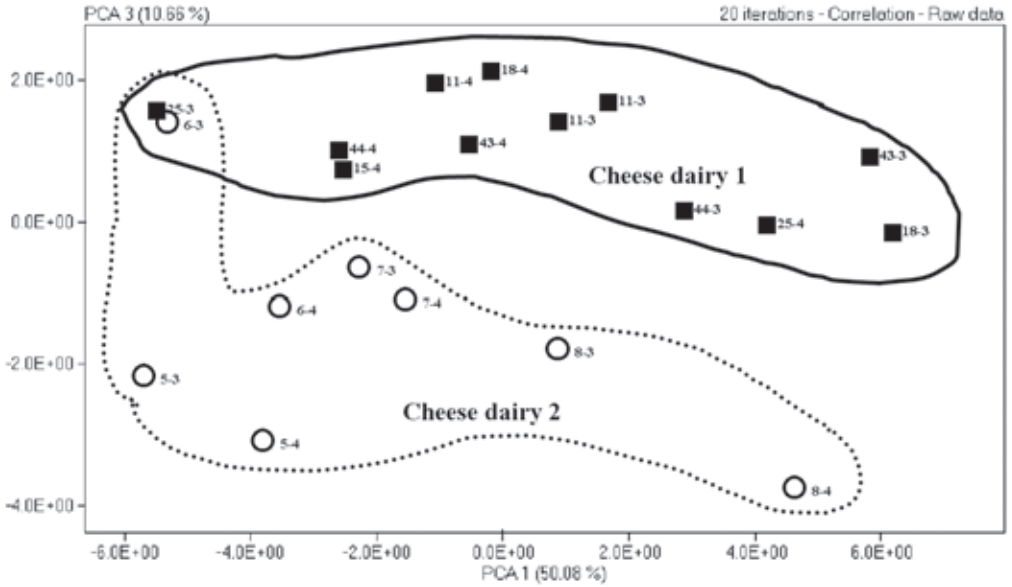

Figure 1: PCA performed with the masses of the volatile compounds produced in milk samples by strains of *Lactobacillus rhamnosus* retrieved from cheese factory 1 and 2. Each sample was analysed twice and the results were confirmed with a second set of samples.

P087 THE ROLE OF MOULDS ON SMEAR-RIPENED CHEESES

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Quite a large number of cheese varieties are characterised by the development of a specific surface microflora. In the context of non-mould ripened cheeses, moulds are mentioned mainly as spoilage organisms that produce mycotoxins. However, besides the well-known cheese varieties with pure desirable mould ripening, there are other cheese varieties where mould growth occurs and can be desirable. These are cheeses with a so-called mixed surface flora (mainly moulds but also smear bacteria) or with a smeared surface (mainly smear bacteria).

Numerous traditional Swiss cheese varieties are smear ripened. In recent years, there has been an increase in the occurrence of stickiness of the smear. During ripening, the smear became bright and viscous like honey. The consequences are an alteration of the typical flavour and a labour-intensive handling of the cheeses.

Our work has clearly demonstrated that the growth of adventitious white mould is not necessarily coincidental or undesirable. The adventitious white mould is part of the natural ecosystem of the smear and contributes greatly to its quality. Practical experience had shown that cheeses with a natural growth of white moulds seldom became sticky. *Trichothecium domesticum*, a white mould, often occurred on the surface of good cheese bodies, but rarely on cheeses a sticky smear. Further analysis showed that this mould is relatively widespread. In culture tests, whether on a model at ALP or directly in practice with our customers, *T. domesticum* had a thoroughly positive effect on the characteristics of the smear. The cheese-makers were very satisfied with the quality of the smear and in most cases noted a reduction in, or the disappearance of the sticky smear defect.

However, it was not known whether the natural white mould is simply one species of mould or whether it consists of several species. Therefore the natural white mould on various smear-ripened cheese varieties was examined. It was found that two moulds are dominating: *Trichothecium domesticum* and *Scopulariopsis spp*. In culture tests all strains of *T. domesticum* had a thoroughly positive effect on the characteristics of the smear and *Scopulariopsis spp*., in contrast, often accelerated the stickiness.

Keywords: moulds, *Trichothecium domesticum, Scopulariopsis spp.*, smear, sticky smear, cheese

P088 DEVELOPMENT OF STARTER AND ADJUNCT CULTURES FOR CHEESE-MAKING IN THE PHARAOH'S LAND

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Lactic Acid Bacteria which are considered to be the most useful microorganisms to society are involved in the manufacture of thousands of fermented foods, they are also beneficial in flavoring foods, inhibiting pathogenic as well as spoilage bacteria in these products.

A total of 2000 isolates LAB were isolated from various dairy products (Raw milk, Cream, Butter, Ras, Domiatti, Kareish and Mish cheese and the following fermented milks:- Laban Rayeb and Zabady) collected from different geographical locations in Egypt.

The pre-identification tests were performed according to morphological characteristics, catalase test, growth at 10 °C and 45 °C, growth in the presence of 6.5 % NaCl and CO_2 production. The isolates were subjected to phenotypic and cell wall protein characterization analyses. Phenotypically, 1006 strains were identified using API 50 CHL and API 20 Strep. The SDS-PAGE technique (Sodium dodecyl sulfate) of whole cell protein was also evaluated for the identification of LAB. Protein fingerprints were registered for 514 strains and compared to a large number of LAB reference strains stored in a database.

The SDS-PAGE technique confirmed 94 % of the API identification results. The identified strains were tested for acidifying, autolytic, aminopeptidase and, antagonistic activities as well as for polysaccharide production.

Selected cultures where then evaluated as starter or adjunct cultures for Ras cheese production in the cheese research laboratory. Higher cheese flavor scores were consistently obtained in Ras cheese made with cultures showing high peptidase and esterase activity and a high rate of autolysis. Development of free amino acids and free fatty acids in Ras cheese were always higher when compared to the cheese made using other starters.

Keywords: lactic Acid Bacteria, Free amino acids , Free fatty acids , SDS-PAGE , API

P089 PRODUCTION OF DIACETYL AND ACETOIN IN WASHED-CURD CHEESE MADE WITH DIFFERENT STARTERS AND ADJUNCT LACTOBACILLI

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Washed-curd cheeses were made with different starters and an adjunct *Lactobacillus plantarum* INF15D to study the influence of the starter on the production of cheese flavour by the adjunct starter. The cheese was made according to the technique used for Edam type varieties, and was held constant. The starters used (Christian Hansen, Hørsholm, Denmark) were citrate-positive mesophilic DL-starters (CH N11 and CH N19) and a citrate-negative mesophilic O-starter (R-704) and a thermophilic starter TCC20. No differences between the cheeses made using different starters were found for microbial counts during ripening.

Previous experiments have shown that the adjunct used has a strong Ser metabolism with the production of a high level of acetate as one of the metabolites. In this experiment, the level of Ser was very low in all cheeses with similar production of acetate, indicating that the growth of the adjunct was good and similar in all cheeses.

In young cheese the concentrations of acetoin and diacetyl were highest in cheese made with CH N19; however, cheese made with R-704 had higher levels than cheese made with CH N11. During ripening, the concentrations of these compounds decreased but the highest concentrations were found in cheese made with R-704. The production of acetoin and diacetyl was lowest in cheese made with the thermophilic starter, TCC20, throughout ripening. In cheese made with the O-starter, diacetyl and acetoin were probably derived from Asp as the content of this free amino acid was reduced in this cheese. The concentration of Asp was less reduced in cheese made with the DL starters. This indicates that as the DL starters produce diacetyl and acetoin from citrate, the degradation of Asp by the adjunct *Lactobacillus plantarum* INF15D is less likely expressed than with the citrat-negative starters.

Keywords: starter, adjunct, aspartate, metabolites

P090 CHARACTERIZATION OF YEASTS INVOLVED IN THE RIPENING OF PECORINO CROTONESE

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Pecorino Crotenese is a traditional cheese produced in a well defined area of Southern Italy. It has been comprehended by European Community (EC) among the foodstuff with Protected Denomination of Origin (PDO). According to the protocol approved by EC it is produced from pasteurised sheep milk by using caprine rennet and natural whey starter cultures. It is ripened at least for 2 months, although it can be commercialised also after 1 year.

During the ripening several microbial populations coexist and/or succeed, including enterococci, micrococci, NSLAB and yeasts. The precise role of these different microbial groups has not yet been defined. In this work yeasts isolated from different aged cheese have been identified and characterized. In particular, the yeast isolates were identified according to traditional physiological tests as well as molecular techniques. The isolates have been further investigated for some characteristics having technological importance.

To evaluate the impact of the yeast on the ripening profile of the cheese, the proteolytic and lipolytic extracellular activities of the isolates have been firstly studied. The isolates have also been studied for their ability to ferment/assimilate lactose, galactose, glucose, citric acid also in the presence of different NaCl concentrations. Moreover, it has been evaluated their aptitude to produce undesired brown pigments from tyrosine and their ability to produce biogenic amines. This last characteristics have been screened using a specific medium for yeasts.

The results have evidenced that yeasts can play an important role in the organoleptic characterization of this traditional cheese. Nevertheless, the ability of some isolates to produce brown pigments and biogenic amines suggests that a specific strain selection is necessary to obtain this typical product without undesirable characteristics.

Keywords: PDO, yeasts, hydrolytic activities, brown pigments, biogenic amines

P091 PHAGE-INACTIVATION IN MILK AND RELATED MEDIA BY THERMAL AND HYDROSTATIC TREATMENT

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(Bacterio-)phages are still a major problem in the field of industrial bacterial fermentations, cheese being one of the most important products affected. Phages are brought into the dairy via raw milk or air and can be spread throughout the plant and taken into the cheese vat by air or carelessly handled whey. Once the starter is infected, phages attack the bacteria and multiply rapidly. Poor product quality, fermentation delay or failure may be the consequences.

Inactivating the phages present in milk or whey has the potential to minimize this risk. This requires knowledge of their inactivation kinetics. Further questions arise such as: which phage strain is most resistant to heat and/or high pressure and can act as an indicator strain for sufficient inactivation? How does the suspension medium influence the rate of inactivation? How can inactivation kinetics be approximated and predicted?

Inactivation experiments were carried out with several commonly occurring lactococcal and streptococcal phage strains. Phage suspensions were heated at 55 to 80 °C, or high-pressure treated at up to 6000 bar. Holding time was varied as appropriate. Phage counts were determined by the plaquetest-method by Adams. In addition, different milk-related dilution media were employed for the experiments to study the effect of specific milk components on phage inactivation.

The kinetic evaluation showed that the order of inactivation reaction was above 1. Thus, inactivation kinetics were approximated by a non-linear regression model. The Arrhenius parameters, velocity rate, k_0, and activation energy, E_A (for heat treatments) and activation volume, $\Delta V^{\#}$ (for pressure treatments) were calculated. Milk increased the thermal resistance of the phage which was attributed to interactions between phages and milk components.

The determined parameters allow prediction of phage inactivation and help to optimize process conditions, depending on the number of phages present and considering the effect of milk components. This becomes more essential when working in cheese-making with milk concentrates.

Keywords: bacteriophage, thermal inactivation, high pressure, kinetic modelling

P092 INFLUENCE OF ADJUNCT CULTURES ON THE EVOLUTION OF DIFFERENT MICROBIAL GROUPS IN SPANISH EWE´S MILK CHEESE

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The object of the present study was to examine the effect of different facultatively heterofermentative lactobacilli (FHL) on Spanish ewe's milk cheese in the evolution of different microbial groups. Besides, a follow up of both adjunct cultures was performed to note their evolution and implantation in the cheese.

Four cheese batches were made: one from raw milk (C), one from pasteurised milk (P), another made from pasteurised milk with an added native *Lb. paracasei* (cit^+) (FC) and a fourth made from pasteurised milk with a different native culture consisting of *Lb. paracasei* (cit^+) + *Lb. plantarum* (cit^-) (FCP). To ensure a correct acidification of the cheese during manufacturing, a commercial starter (*Lactococcus lactis*) was added to every vat. The cheeses were ripened at 12 °C and analysed at 1, 15, 30, 120 and 240 days of ripening.

The two different adjunct cultures, had a good implantation in the cheese, and maintained levels of around 10^7 cfu/g of cheese throughout the whole ripening process. However and despite having added a similar amount of *Lb. paracasei* and *Lb. plantarum* in batch FCP, the first species was predominant during the whole maturing process.

In the batches made with lactobacilli as adjunct cultures, the decrease of native enterobacteriaceae and enterococci was faster so we could deduct a rivalry between the lactobacilli used as adjunct cultures and those micro-organisms. Besides, the inhibition effect was higher when utilising *Lb. paracasei* + *Lb. plantarum* as adjunct culture.

The levels of *lactococci* were high in the batches made with FHL as adjunct cultures, so we can think of a synergic effect between both micro-organisms.

Despite having used *Lb. paracasei* (Cit^+), no differences were observed in lactose, diacetyl, acetoin and organic acids (formic, citric, lactic and acetic). The lactococci present in the starter culture displaced the lactobacilli and were mainly responsible for the metabolism of the carbohydrates.

Legend: cit^+ = citrate positive; cit^- = citrate negative

Keywords: ewe milk cheese, adjunct culture, cheese ripening

P093 RIPENING OF WHITE-PICKLED CHEESE

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White cheese is the most commonly consumed cheese in Turkey. White cheese is a semi-hard pickled cheese with a minimum ripening period of 30 days. Proteolysis and lipolysis in white-pickled cheese produced from pasteurised cow's milk were measured during refrigerated storage for 3 months. Proteolysis was followed by measurements of amounts of water-soluble N, TCA-soluble N and free amino acids by TNBS method. Water-soluble N and TCA-soluble N levels increased by two-fold and amounts of amino acid N increased by five-fold after storage for 3 months. Proteolysis was attributed to the activities of rennet and bacteria. Low level of lipolysis was observed during storage. Even though white-pickled cheese contained high moisture, its low pH of 4.6 possibly reduced the rates of proteolysis and lipolysis.

Keywords: white cheese, ripening, proteolysis, lipolysis

P094 MICROBIOLOGICAL, BIOCHEMICAL AND ORGANOLEPTIC PROFILES OF SERRA DA ESTRELA CHEESE MANUFACTURED WITH WILD STRAINS OF LACTIC ACID BACTERIA

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Experimental cheeses were manufactured via inoculation with wild strains of *Lactococcus lactis* subsp. *lactis* strain ESB110019 and *Lactobacillus plantarum* strain ESB5004. Those two strains had been isolated from the adventitious microflora of typical Serra da Estrela cheese. The evolution in viable counts of the main microorganisms (viz. lactic acid bacteria, *Enterobacteriaceae*, staphylococci and yeasts), as well in secondary proteolysis (WSN, 2 % TCASN, 12 % TCASN and 5 % PTASN) and lipolysis (fat acidity) were

monitored throughout ripening time (over a 63 d-period). The sensorial features of the 63 d-old cheeses were also assessed. Cheeses manufactured with starter showed significantly lower levels of viable *Enterobacteriaceae* than those manufactured in its absence, but viable counts of enterococci did significantly increase following addition of *L. lactis*. Proteolysis and lipolysis were not significantly affected by the lactic acid bacteria tested when compared to the control, but *L. lactis* played a significant role toward increasing the 2 % TCASN content of cheeses. The scores for flavor and texture of the control cheeses were somewhat above those for the experimental cheeses manufactured with starter.

Keywords: ewe's milk cheese, starter, microbiology, proteolysis, lipolysis

P095 SURFACE MICROFLORA OF LIVAROT, A TRADITIONAL SMEAR RIPENED CHEESE

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Livarot is a French smear-ripened cheese manufactured in a restricted area in Normandy. The ripening of such cheeses is characterised by the development of a succession of largely undefined microbial communities on the surface. Generally, yeasts dominate the surface initially because they are acid tolerant and metabolize the lactic acid produced by the starter. So, the pH increases to 5–7 allowing growth of bacteria of the genera *Brevibacterium* and *Corynebacterium* to occur. The ecology of the micro-organisms involved in the smear is complex and not very well understood.

In this study, we have observed the composition of the surface microflora of Livarot from three different dairies at three points during ripening. At each point, we isolated 50 yeasts and 50 bacteria. Yeasts were dereplicated by Fourier Transform Infrared Spectroscopy (FTIR spectroscopy), bacteria were dereplicated by Repetitive Extragenic Palindromic PCR (REP-PCR). Representative yeasts and bacteria from different clusters were identified by a polyphasic approach, including phenotypic and genotypic tests.

Eight different genera of yeasts were identified: *Galactomyces*, *Yarrowia*, *Kluyveromyces*, *Debaryomyces*, *Candida*, *Torulaspora*, *Pichia* and *Cryptococcus*.

Thirty four per cent of the bacteria were Gram negative, 15.5 % were LAB, and 45.5 % were Gram positive of the genera: *Arthrobacter*, *Brevibacterium*, *Brachybacterium*, *Corynebacterium*, *Microbacterium*, *Staphylococcus*, *Leucobacter* and 6 % were not classified (description of new taxa is in progress).

Keywords: biodiversity, cheese, Livarot, microflora, surface

P096 HPLC ANALYSIS OF THE PIGMENTS PRODUCED BY THE MICROFLORA ISOLATED FROM ‚PROTECTED DESIGNATION OF ORIGIN' FRENCH RED-SMEAR SOFT CHEESES

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Red-smear ripened soft cheeses are characterised by their orange-red colour. This colour is due to the development of cheese ripening microflora which produces mainly carotenoids. From four types of cheeses (Livarot-Reblochon-Munster-Epoisses) with protected designation of origin, 114 different strains have been isolated. They are constituted of *Brevibacterium linens*, *Micrococcus sp*, cocci and coryneforms. 67 out of those 114 strains have been selected for their colour (orange or yellow) and their methanolic extracts analysed by HPLC. Only four different chromatograms were obtained from the analysis of these 67 strains. All orange bacteria have the same HPLC profile as *Brevibacterium linens*, known to produce 3 aromatic carotenoids (isorenieratene, 3-hydroxyisorenieratene and 3,3'-dihydroxyisorenieratene). The three different chromatograms of yellow bacteria seem to be composed of isomers of the same molecule. This study allows us to obtain pigment fingerprints of bacteria that occur in the microflora of red-smear ripened soft cheeses, as part of the data that could be used to identify those microorganisms. In our future works, we will investigate the relationship between those pigments produced and cheese rind coloration.

Keywords: cheese, colour, bacteria, carotenoid

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P097 EVALUATION OF EYE AND SPLIT FORMATION IN SWISS TYPE CHEESE DURING RIPENING

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During the ripening of Swiss type cheese – apart from classical eye formation – split and crack formation may occur inside the cheese. As one possible cause the late fermentation of proteolytically formed aspartate by aspartate-fermenting propionibacteria is assumed. For the evaluation of this defect the eye and split formation was examined by means of digital photography in several large scale productions of Swiss type cheeses, which were produced with three different combinations of ripening cultures and subsequently computer-assisted image evaluation was applied. The received results were compared with the results of the enzymatic determination of aspartate and ammonia as well as the contents of relevant organic acids. The chemical analyses were matrix-dependently validated and afterwards carried out for four month at selected times during cheese ripening.

The results concerning the chemical ripening parameters succinate, acetate and propionate proved as particularly expressive and therefore could be referred to for the discrimination between the different culture combinations. They pointed out that obviously in certain combinations besides the classical propionic acid fermentation also the metabolic Wood Werkman reaction took place.

Comparing the eye and split formation an inverse proportion was determined concerning the areas of the eyes and splits, which proves that the formation of splits is predetermined simultaneously with the eye's starting point at an early stage of ripening .

Depending upon the production variant and referring to the culture combinations the observed splits correlated differently with the aspartate content. However the ammonia concentrations determined during ripening – regarded as a parameter of the aspartate degradation – did not show a significant correlation to the other determined ripening parameters and to the split formation.

Keywords: Swiss type, split, eye, organic acid, image evaluation, texture defect

P098 EFFECT OF SIMULATED CHEESE CONDITIONS ON THE PHYSIOLOGICAL STATE OF STARTER AND NON-STARTER LACTIC ACID BACTERIA

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Lactic acid bacteria have been observed to exist in a number of different physiological states in cheese during ripening, including viable, viable but not culturable (VBNC), spheroplasts, permeable and autolysed. The tendency for different strains of *Lactococcus lactis* to exist in either the viable or autolysed state has been shown to have a significant effect on cheese ripening. The purpose of this work was to determine whether different strains of *L. lactis* and *Lactobacillus casei* differ in the proportion of their cells that exist in the different physiological states and whether differences in the ion content of the aqueous phase affect these proportions using a simulated cheese environment. One strain of *L. lactis* ssp. *lactis,* three strains of *L. lactis* ssp. *cremoris* and eight strains of *L. casei* were assessed during a one month period at 10 °C for the proportion of their cells in the viable, VBNC, permeable and autolysed states. The simulated cheese environment consisted of either 50 mM sodium acetate buffer (pH 5.0) or a milk salts solution adjusted to pH 5.0 with lactic acid. Both solutions also contained 3.5 % NaCl. For the *L. lactis* strains the proportion of cells in the different states differed significantly at day 7. However, by day 28 all strains were similar in having most cells in the permeable state and only differing slightly in the number of autolysed cells. In contrast, the *L. casei* strains showed a much greater strain-to-strain difference in the proportion of cells in the different states at 28 days with no one state consistently dominating. Comparison of strain behavior in the different buffer systems showed that the ionic environment had little effect on the physiological state of the *L. lactis* strains, but had a significant effect on the viable state of the *L. casei* strains.

Keywords: *Lactococcus lactis, Lactobacillus casei*, physiological state, simulated cheese

P099 PRODUCTION OF PROBIOTIC SOFT CHEESE MADE FROM GOAT'S MILK

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In this paper the probiotic soft cheese was produced from goat's milk with ABT-4 starter culture (Chr. Hansen's). Goat's milk is proven as easier to digest, due to smaller milk fat globules, higher albumine content and lower casein content compared to cow's milk. Since goat's milk differs in technological properties from cow's milk it was necessary to develop production technology that would provide soft

cheese with good sensory quality. Goat's milk gives gentle curd therefore it was necessary to add rennet to get firmer coagulum. Two types of cheeses were produced, full fat cheese from milk with 3.2 % fat, and low fat cheese from milk with 1 % fat. Both cheese types were produced by the same technology. Firstly, the fat content in the milk was standardised, then milk was pasteurised at 65 °C/30 min., inoculated with 2 % of ABT-4 culture and with different percentage of rennet, incubated on 40 °C/7.5 hours, drained over night and then packed and stored at 8 °C. Cheese without added rennet was produced as a control sample. The sensory scores and bacterial count were determined after 1, 7 and 14 days of storage. The best sensory scores were given to the low fat cheese produced with 0.01 % of rennet. All cheese types had high grades and spoilage was not detected after 14 days of storage. The viable count showed that the product has more than 10^6 cfu/ml during 14 days of storage. In the overall bacterial microflora *Streptococcus thermophilus* accounts about 38%, *Lactobacillus acidophilus* around 28% and *Bifidobacterium bifidum* 34%.

Keywords: soft cheese, goat's milk, probiotic, production

P100 *STREPTOCOCCUS MACEDONICUS* ACA-DC 198. GROWTH AND BACTERIOCIN PRODUCTION UNDER CONDITIONS SIMULATING KASSERI CHEESE TECHNOLOGY

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The aim of the present study was to assess the growth and the bacteriocin production of *S. macedonicus* ACA-DC 198 under conditions simulating Kasseri cheese technology. Controlled fermentations were performed in milk (10 %), containing 0.3 % yeast extract, at constant pH 5 and 6, in the presence of NaCl (0, 2 and 4 %) and at 25, 40 and 55 °C. Growth was assessed by the agar plating method and the NaOH consumption and bacteriocin production by the agar well diffusion assay.

Optimal growth and bacteriocin production were recorded at 40 °C, pH 5 and 6, in the absence of NaCl. At 2 % NaCl, growth was slightly reduced, independently of the pH. However, the negative effect on bacteriocin production was more evident. At 4 % NaCl, neither growth nor bacteriocin production was observed over a period of 100 h. At 25°C, growth was delayed compared to 40 °C, but strain reached similar final populations. Both the pH and the NaCl concentration affected growth and bacteriocin production. At 55 °C, a reduction of living cells was observed during the first hours of fermentation, ranging from < 1 log cfu/ml (0 and 2 % NaCl; pH 5 and 6) to 2 log cfu/ml (4 % NaCl; pH 5 and 6). Afterwards, *S. macedonicus* ACA-DC 198 recovered almost completely. However, neither growth nor bacteriocin production was observed over a period of 100 h.

When growth was performed under conditions simulating the temperature program applied during Kasseri cheese preparation, bacteriocin was produced during the initial steps of the process, with the temperature ranging from 16 to 45 °C and the bacteriocin activity remained stable after the heat treatment at 75 °C. The strain itself not only survived the 75 °C treatment (2.5 log reduction), but it showed almost full recovery when the temperature subsequently increased up to 25 °C.

Keywords: *S. macedonicus*, fermentation, growth, bacteriocin, Kasseri

P101 *STREPTOCOCCUS MACEDONICUS* ACA-DC 198 AS ADJUNCT STARTER IN KASSERI CHEESE PRODUCTION

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The aim of the present study was to assess the growth of and the bacteriocin production by *S. macedonicus* ACA-DC 198 during Kasseri cheese production. Kasseri cheese was prepared from ewe's milk using *S. macedonicus* ACA-DC 198 as adjunct starter. Microbiological analyses and follow up of both growth and bacteriocin production of *S. macedonicus* ACA-DC 198 were performed over a period of 90 days.

By using selective growth medium (M17 agar, supplemented with 100 μg/ml streptomycin) as well as species-specific PCR it was shown that *S. macedonicus* ACA-DC 198 survived cheese cooking at 75 °C, and it was present in cheese up to the 90th day of ripening. Extraction using either 2 % (w/v) sodium citrate or 0.02 M HCl solution revealed the presence of *S. macedonicus* ACA-DC 198 bacteriocin (macedocin) throughout ripening.

Regarding the microbiological characteristics of the cheese, significant differences were observed to the control for the counts of thermophilic bacilli, thermophilic cocci and mesophilic cocci at 60 days of ripening. Finally, *S. macedonicus* ACA-DC 198 affected positively the overall sensory profile of Kasseri cheese, even though with no significant differences compared to the control.

Keywords: *S. macedonicus*, Kasseri, PCR, bacteriocin

P102 EFFECTS OF ADJUNCT STARTER PROPERTIES ON CHARACTERISTIC QUALITY ATTRIBUTES OF HARD CHEESE

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A large number of microorganisms is associated with cheese ripening and the formation of texture and flavour. Among them, the facultatively heterofermentative lactobacilli (FHL) are discussed as adjunct starters to improve cheese appearance or to reduce cheese defects such as late fermentation.

In pilot-plant productions Emmental and Bergkäse were produced from pasteurised milk with standard starters and strains of FHL (*Lactobacillus casei* subsp. *casei* and *L. rhamnosus*), selected with respect to their ability to utilise citric acid. Cheese microbiology, chemical and biochemical parameters were monitored throughout ripening and mature cheeses were subjected to mechanical and sensory analysis.

The development of the FHL showed maximum values of 10^8 cfu/g in 2–4 weeks old cheeses and viable counts of 10^7 cfu/g in mature cheeses. When added, Bergkäse showed viable enterococci counts remaining almost constant (4×10^3 cfu/g), whereas in Emmental the viable counts diminished to not detectable in mature cheeses. Regarding propionibacteria, a more rapidly development was found in Emmental made with the citrate (-) *L. rhamnosus,* leading to significantly higher values of propionic acid compared to Emmental made with the citrate (+) *L. rhamnosus* strain. In Bergkäse, citrate (+) FHL strains increased the number of eyes due to the corresponding formation of carbon dioxide, resulting in appearance properties which are in accordance with the consumer's expectance. Texture and sensory assessments revealed, that the FHL do not show any negative effects on the organoleptical properties of the cheeses.

Keywords: Swiss-type cheese, adjunct starters, FHL

P103 PRODUCTION OF EMMENTAL CHEESE WITH ADJUNCT STARTERS WITH VARYING DEGREE OF AUTOLYSIS

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The microbial flora of traditional raw milk cheeses is mainly determined by starter micro-organisms and the microflora of the vat milk. Besides others, the indigenous milk microflora consists of non-starter lactic acid bacteria (NSLAB), including facultatively heterofermentative lactobacilli (FHL). FHL have been identified to influence cheese quality significantly by improving cheese appearance or aroma, or by reducing the risk of cheese defects such as secondary fermentation.

FHL vary with respect to several properties, including their potential towards autolysis. Cell lysis results in the liberation of intracellular enzymes, which presumably enhance peptidolytic reactions in the cheese mass. Using Emmental cheese as an example, the study aimed to determine whether the autolysis of FHL adjuncts shows interactions with the cheese microflora, flavour and texture formation.

Although FHL were not detectable in raw milk, FHL developed up to 2.5×10^8 cfu/g in all mature cheeses. In cheeses with FHL adjuncts, the evolution of propionibacteria was slightly delayed, as was the concentration of propionic acid and the cheeses showed a reduced number of eyes. A significant lower number of enterococci were observed in cheeses with FHL adjuncts showing a high level of autolysis. As the selected FHL strains were able to utilise citric acid, its content decreased to < 1 mmol/kg within the initial phase of maturation. Emmental cheese with fast autolysing FHL strains showed significantly higher concentrations of soluble nitrogen fractions and sensory analysis revealed higher scores for smell and aroma.

Keywords: emmental cheese, adjunct starters, FHL, autolysis

P104 ALTERED PROTEOLYSIS OF MILK PROTEINS BY GENETICALLY MODIFIED *LACTOBACILLUS HELVETICUS*

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The proteolytic system of dairy lactic acid bacteria is important both for the growth of these bacteria in milk and for the development of the flavour and texture of fermented milk products. In cheese manufacturing, various strains of *Lactobacillus helveticus* are generally used as adjunct cultures to reduce bitterness, improve flavour development and accelerate the ripening process.

Studies of peptidase mutant strains have shown that the optimal growth of lactic acid bacteria in milk depends on the cooperative action of several peptidases with different specificities for the release of amino acids from peptides derived from milk proteins. Free amino acids can be utilized for new protein synthesis and generation of metabolic energy. In cheese manufacturing, small peptides and free amino acids are the precursors of aroma compounds.

In this study, the significance of a *Lb. helveticus* broad-specificity aminopeptidase (aminopeptidase N) and a proline-specific aminopeptidase (X-prolyl dipeptidyl aminopeptidase) for the proteolysis of milk proteins was investigated. Peptidase mutants of *Lb. helveticus* were constructed by molecular methods and grown in pasteurized skimmed milk under anaerobic conditions. The degree of proteolysis and profile of generated peptides were determined in samples taken from the grown cultures.

Keywords: cheese, proteolysis, peptide, lactic acid bacteria

P105 CONTRIBUTION OF *GEOTRICHUM CANDIDUM* TO THE PROTEOLYSIS OF SOFT CHEESE

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The presence of surface flora gives a specific appearance to cheese and produces typical aroma and taste. *Geotrichum candidum*, a yeast-like fungi, is found at the surface of many soft and semi-hard cheeses where it may have a role during the ripening through its biochemical activities. Because the proteolytic system of *G. candidum* remains quite unknown, in contrast to its lipolytic system, we estimated *in situ* the proteolytic activity of *G. candidum*.

The strain *G. candidum* Geo17 was used to manufacture model soft cheeses. A control cheese without surface flora, thus containing only *L. lactis* as maturing agent, was also manufactured. Cheeses were withdrawn throughout 27 days of ripening. Extent and nature of the proteolysis were determined at the surface of the cheeses.

β-casein was not hydrolysed in control cheese throughout ripening, while the intensity of the β-casein band decreased from first days in cheese manufactured with *G. candidum* and remained constant after 12 days. α_{s1}-casein was weakly degraded in control cheeses. But α_{s1}-casein hydrolysis was higher and occurred sooner in cheese containing *G. candidum*. The amount of pH 4.6 soluble nitrogen, i.e. large and medium fragments of caseins, first decreased in cheese with surface flora, suggesting their consumption by *G. candidum*. After 8 days, *G. candidum* produced soluble nitrogen as a consequence of casein hydrolysis. The amount of soluble nitrogen increased largely until 15 days and increased slightly thereafter. In contrast, *G. candidum* produced most peptides and free amino acids from day 12 to the end of the ripening. Particularly, *G. candidum* produce significantly high concentrations of glutamic acid, glutamine, proline, alanine, histidine, leucine and lysine. *G. candidum* contributes to primary and specially to secondary proteolysis of soft cheese. But the corresponding proteolytic enzymes are unknown.

Keywords: *Geotrichum candidum*, proteolysis, soft cheese

P106 UP-REGULATION OF A 42 kDa ACID STRESS PROTEIN IN *LACTOBACILLUS PARACASEI* STRAINS

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Eight *Lactobacillus* strains were screened using microtitre tray growth assays for their ability to initiate growth over a wide range of pH values (1.6 to 8.2). *Lb. paracasei* strain VUP 12006 demonstrated the highest μ_{max} (0.23 h^{-1}) of the eight strains tested at its optimum pH and the widest growth range (pH 3.1 to 8.2). Optimum conditions for the growth of strain VUP 12006 were then determined using fermenters with set pH and temperature control and were found to be pH 6.3 ± 0.2 and 37 °C ± 0.5.

At pH 4.3, the μ_{max} was 50 % of the value seen at pH 6.3. Cellular proteins were prepared following growth at pH 4.3 and 6.3 and analysed by 1D SDS-PAGE for cytosolic and cell-surface fractions. The most significant change in protein profiles following growth at pH 4.3 was the up-regulation of a 42 kDa protein, which was found to be membrane- or cell-surface-associated. This up-regulation was observed when the culture was grown continuously at pH 4.3 and when the pH was changed to pH 4.3 after two hours growth at pH 6.3. Up-regulation of a similar sized protein was also seen in *Lb. paracasei* 1180 (isolated from Canadian cheddar cheese) and *Lb. paracasei* 292 (*Lb. paracasei* type strain NCDO 151), although this was less marked in strain 292. However, under identical growth conditions, similar observations were not made for two strains of *Lb. acidophilus* (strains VUP 12001 and VUP 12007), which suggest a species-specific response occurs.

There was sufficient separation on 1-D SDS-PAGE of the cell-surface proteins prepared by SDS extraction to enable N-terminal sequence analysis of the 42 kDa protein. The sequence obtained (Asp, Thr, Ser, Asp, Ser. Ile, Ala, Ser, Asn, Lys, [Ser/Gln/Asp], Glu, Thr, Asn, Ala, Leu, Leu, Lys, Gln, Ile) was submitted for *in silico* analysis using BLASTn and was found to match positions between 9501 and 9560 of scaffold 3 of the *Lb. casei* draft genome.

Keywords: *Lb. paracasei*, acid stress

P107 YEASTS AS ANAEROBIC ADJUNCT CULTURES IN CHEESE

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Yeasts are traditionally used in surface ripened cheeses. The objective of this study was to identify the ripening potential of yeasts in anaerobically ripened cheeses. We hypothesised that even without growth in the cheese, the enzymes already present in the yeast adjunct would influence ripening. Therefore the conditions of culture preparation and the level of culture addition, which would influence the types and levels of enzymes present, could be important. Here we are reporting the effect of culture conditions on lipase activities of *Geotrichum candidum* and *Yarrowia lipolytica* and the effect of the yeast adjuncts at different levels on ripening of 1 kg blocks of dry salted, washed curd, semi-hard cheese. The cheese also contained *Propionibacterium*, *Lactobacillus fermentum* and *Lactobacillus rhamnosus* as bacterial adjuncts.

G. candidum and *Y. lipolytica* were grown in glucose and safflower medium as shake or static cultures. The lipase activity of *G. candidum* was totally extracellular whereas *Y. lipolytica* showed extracellular, intracellular and cell-bound lipase activity. We selected a 24 h shake culture of *G. candidum* and a 192 h static culture of *Y. lipolytica,* both grown in safflower oil medium, to use as cheese adjuncts. Both cultures had high lipase activity (1.6 and 5.9 mmol/min/mL culture, respectively) as determined by activity on the chromogenic ester substrate *p*-nitrophenyl butyrate.

The concentration of the free fatty acids in cheese increased with the increase in the level of yeast adjunct, e.g., 3, 15, 20, and 36 mg/g cheese in cheeses with 0, 0.2, 1 and 5% of *Y. lipolytica,* respectively, after 4 months of ripening at 20 °C. Higher ratios of oleic plus linoleic acids to palmitic plus stearic acids in yeast cheeses (1.2–1.7:1) compared with those in control cheese (0.7:1) indicated the presence of a lipase selective for unsaturated fatty acids. Higher levels of ethyl esters of fatty acids and ketone compounds were detected in yeast cheeses.

We conclude that yeasts as anaerobic adjuncts can influence the ripening of cheese and that the effect depends on the culture preparation and level of culture use.

Keywords: *Geotrichum candidum*, cheese, lipase, *Yarrowia lipolytica*

P108 EFFECT OF METHOD OF MANUFACTURE, CULTURE/ENZYME SYSTEMS AND RIPENING TEMPERATURE ON THE BIOCHEMICAL EVENTS DURING CHEDDAR CHEESE RIPENING

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The quality of Cheddar cheese during ripening is greatly influenced by method of manufacture, type of culture and ripening temperature and the objectives of this study were to determine effect of these factors on the quality and ripening of Cheddar. Three replicate experimental cheesemaking trials (by both milled and stirred curd methods) were conducted using specific culture systems supplied by four commercial culture companies. On each cheesemaking day, two 625 kg lots of milk were made into Cheddar cheese with one culture system by each method. Cheese curds in 18.2 kg blocks were pressed overnight in a vacuum chamber with 24 mmHg for first 60 min. Cheeses were ripened at 2 or 8 °C and sampled at intervals for starter and non-starter lactic acid bacteria (NSLAB), and proteolysis. Volatile flavor compounds during ripening were also determined by gas chromatography-mass spectrometry. Most of the compositional attributes except for salt and protein contents ($P < 0.01$), growth of starter or NSLAB were not significantly affected ($P > 0.01$) by the

method of manufacture. Proteolysis and flavor compounds was influenced by method of manufacture. The culture system affected cell density of NSLAB. Different volatile compounds were produced by different culture systems in the cheeses during ripening, however, compounds such as 2-butanone, 2-3-butanedione and acetic acid were produced by all cultures but with different concentrations.

Keywords: Cheddar cheese ripening, proteolysis, volatile compounds

P109 *LACTOBACILLUS HELVETICUS* AS ADJUNCT CULTURE IN LOW-FAT SEMI-HARD CHEESE

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Semi-hard, low-fat cheese often fails to develop appreciated flavour and texture. However, successful cheese varieties without smeared surfaces and less than 10 % fat have been developed by accelerating the amino acid release from cheese peptides using heat treated *Lactobacillus helveticus* cells with a high ratio of aminopeptidase to protease activities. In this work the effect of two viable *Lb. helveticus* cultures were tested in semi-hard cheese with 13 % fat: LHB02 (Chr. Hansen A/S) with mixed strains selected for their aminopeptidase to protease ratio, and LH32 (Chr. Hansen A/S) that is a single strain culture related to CNRZ32. Pilot plant cheese making experiments were repeated three times at different days, and at each day three cheese trials were made in 1000 L processing tanks, *i.e.* one with each adjunct and one control without any adjunct.

Sensory analysis showed effects of both cultures on flavour. Best score was obtained with LHB02 that also removed bitter taste, in contrast to LH32. In cheese with LHB02 a weak nutty flavour developed, while buttery (diacetyl) flavour dominated in those with LH32. Consistency was slightly improved and amino acid release was accelerated by both cultures. An accelerated breakdown of β-casein was obtained in cheeses added CH32, but no other influence on primary breakdown of the caseins was shown using capillary electrophoresis (CE). Impact of the *Lb. helveticus* cultures on peptide composition was studied during ripening using RP-HPLC and LC-MS, and enzyme activities specific for the adjuncts were confirmed.

It was concluded that cultures of selected strains of *Lb. helveticus* could be used as adjuncts in semi-hard low-fat cheese to add buttery and nutty flavours without bitter defects.

Keywords: Low-fat cheese, *Lactobacillus helveticus*, cheese ripening, flavour development

P110 MICROBIOLOGICAL QUALITY OF EWE'S MILK CHEESE MADE WITH LAMB RENNET PASTE

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Lamb rennet pastes are traditional coagulants used primarily in Mediterranean countries to make ewe's milk cheeses, many of which are highly- appreciated traditional products protected by official Denominations of Origin. Their use develops the characteristic piquant flavour of some Italian or Spanish cheeses. Cheese makers usually prepare their own rennet pastes from lamb stomachs, following time-honoured recipes. Because these rennets are not standardized, both their enzymatic content and their microbiological quality can vary substantially. In the present work, we examined the microbiological quality of 31 samples of artisanally-produced lamb rennet pastes (22 from Spain and 9 from Italy), and of the cheeses made with 5 of them, to determine their hygienic quality. The following microbiological parameters were studied in rennet pastes, milk, whey and rennet extract: total aerobic mesophiles, enterobacteria, *E. coli, S. aureus,* sulphite-reducing clostridia, moulds and yeasts, and salmonella. Cheeses were made in 5 farms (2 fabrications each), and samples were taken for analysis after 1, 60 and 150 ripening days. The same microbiological parameters were determined in cheeses, except that coliforms were determined instead of enterobacteria. Enterobacteria, *E. coli*, and salmonella were not detected in any of the rennet pastes. Average log values for rennet pastes were: total aerobic mesophiles, 4.04 ± 0.61 cfu/g; *S. aureus*, 0.79 ± 0.98 cfu/g; clostridia, 2.13 ± 0.93 cfu/g; moulds and yeasts, 1.28 ± 1.12 cfu/g. Average log values in one-day-old cheeses were: mesophiles, 9.68 ± 0.03 cfu/g; coliforms, 4.07 ± 0.96 cfu/g; *E.coli*, 2.65 ± 1.63 cfu/g; clostridia, 0.24 ± 0.41 cfu/g; *S. aureus*, 1.88 ± 0.75 cfu/g, and moulds and yeasts, 3.58 ± 0.43 cfu/g. The levels of all microorganisms analyzed decreased dramatically as ripening progressed, and after 150 days *E. coli* and clostridia were not detected. We conclude that cheeses manufactured with lamb rennet pastes present a good hygienic quality by the time of their commercialization.

Keywords: lamb rennet paste, microbiological quality, ewe's milk cheese

P111 CHARACTERISATION OF A PEPTIDASE RICH SUB-PROTEOME FROM *LACTOBACILLUS HELVETICUS* ITG LH1 GROWN IN MILK

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Lactobacillus helveticus strains are among the most nutritionally fastidious lactic acid bacteria, as they present numerous amino acid auxothrophies. In order to assure its nutritional requirements when grown in milk, *Lb helveticus* counts on a potent proteolytic system capable of producing short peptides and liberating amino acids from the casein matrix. The uses of *Lb helveticus* in the dairy industry include a broad range of applications, from cheese ripening to the preparation of fermented milk products with biologically active peptides.

Despite their great technological interest, very few proteomic studies concerning *Lactobacilli* have been found in the literature, and even fewer concerning strains grown in milk. The aim of this work was to characterise the proteome of a cell free extract of *Lb helveticus* ITG LH1 grown in milk, paying special attention to its particular proteolytic system.

A two step-chromatography methodology, based on ion exchange and affinity chromatography, was developed for the preparation of a peptidase-rich sub-proteome. For that purpose several affinity chromatography columns were tested and among them a HiTrap™ Chelating column was selected. The characterisation of this sub-proteome was performed by 2D-electrophoresis and MALDI-TOF/MS peptide mass fingerprinting. This methodology could be applied to further comparative or dynamic studies, such as evaluating peptidase expression modifications under different stress conditions or monitoring the evolution of the proteolytic system of a particular strain throughout several technological treatments.

Keywords: 2D-electrophoresis, proteolytic enzymes, *Lactobacillus helveticus*

P112 QUANTIFICATION OF LACTIC ACID BACTERIA IN UNDEFINED STARTER CULTURES WITH qPCR AND FISH

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In many dairies undefined mixtures of lactic acid bacteria are the most extensively used cheese starter cultures, particularly in European countries. While undefined cultures offer significant advantages in terms of their technological properties such as aroma formation and phage resistance, the flora composition is frequently not well characterized. Conventional plate counting based on selective media has been the chosen method in most microbiological laboratories to analyse the composition of these cultures. One of the major drawbacks of cultivation-based assays is its rather high time-consumption of about 5 days. In this study, quantitative PCR was used to enumerate *Leuconostoc* sp., *Lactococcus lactis* subsp. *cremoris*, and *Lactococcus lactis* subsp. *lactis* in undefined multi strain PROBAT™ starter cultures. Both, Molecular Beacons and Taqman probes were evaluated to detect specific PCR amplicons. A multiplex assay was developed to quantify subpopulations relative to total bacterial DNA. The technique was compared with whole-cell fluorescence in situ hybridization (FISH) and conventional cultivation-based enumeration.

Keywords: undefined starter cultures, Real-time PCR, quantitative PCR, FISH

P113 NEW SCREENING STRATEGIES FOR CHEESE STARTER CULTURES

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The enzymatic properties of starter cultures during the cheese ripening process are important factors for the preparation of cheese of the desired quality. In addition, the enzymatic properties including a high acidification activity determine the duration of the ripening process. Thus, effective starter cultures are important components for an efficient cheese production.

In the present study, new microplate-based screening assays for starter cultures with defined properties were developed. Fluorescence and absorbance measurement assays for lipolytic, proteolytic, and aminopeptidase activity were developed and adopted for both liquid and solid samples. More than twenty different single strains and the undefined multi strain culture PROBAT™ 505 were grown on different media. The changes of lipolytic, proteolytic, and aminopeptidase activities during simulated ripening and storage over four weeks were studied. Enzyme activities were compared with sensory properties over the same period. In addition, enzymatic and sensory

properties of individual strains during cheese ripening were compared with their genetic affiliation based on their 16S rDNA- and lactate dehydrogenase sequences as well as their fAFLP-DNA fingerprints.

In sum, the developed methodologies allow a more rapid and straightforward screening of new strains for cheese ripening.

Keywords: starter culture, enzyme screening assay, genetic properties, correlation with sensory properties

P114 MICROBIOLOGY OF GUBBEEN, AN IRISH SURFACE-RIPENED CHEESE

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Systematic studies of the composition of the bacteria and yeast microflora of surface-ripened cheeses during ripening are lacking. The dominant bacteria (50 or 80 isolates at each time point) and yeasts (30 or 50 isolates at each time point) of 4 batches of Gubbeen cheese after 4, 16, 23 and 37 days ripening were isolated on PCA containing 5 % salt and YGC, respectively. All bacteria were catalase positive, Gram positive rods or cocci and were dereplicated by PFGE. A representative of each band pattern was identified by rep-PCR and the yeasts by Fourier Transform Infra Red (FTIR) spectroscopy. The bacteria included *Staphylococcus saprophyticus, S. equorum, S. epidermidis, Brevibacterium aurantiacum, Corynebacterium casei*, *C. mooreparkense, C. variabile, C. flavescens, Microbacterium gubbeenense* and a new species of *Agrococcus* while the yeasts included *Debaryomyces hansenii, Candida catenulata, Clavispora lusitaniae* and, occasionally, *Trichosporon ovoides, Can. parapsylosis, Can. intermedia and Pichia guilliermondii.* All microorganisms were not found in every batch of cheese but staphylococci were the dominant bacteria on day 4 and coryneforms on days 16, 23 and 37 indicating a progression in the bacterial composition. Generally there was only a small variation in the yeast composition at each sampling point, except for *D. hansenii* in Batch 1, which showed a decrease with time. It was concluded that there was significant inter-batch but little intra-batch variation in the microbial composition, except for the appearance of staphylococci on day 4 and their subsequent disappearance. Several strains of *B. aurantiacum* were found.

Keywords: smear cheese, yeasts, coryneforms

P115 METHOD FOR THE CHARACTERISATION AND EVALUATION OF CULTURES FOR THE USE IN SEMI-HARD CHEESE

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Investigations of new, selected microorganisms influencing the characteristics of cheese are time-intensive and expensive. A standardised characterisation method, simulating biochemical ripening processes in a lab scale, would give a competitive edge. Thus, the aim of this study was to develop and test a simple, fast and reproducible method to determine the influence of mesophilic cultures on the quality of semi-hard cheese.

Protein-powder, full cream, citric buffer and NaCl were blended to a homogeneous concentrate (protein to fat ratio 1; dry matter 30 %). The concentrate was inoculated with commercial or non-commercial cultures (3 concentration levels), coagulated, incubated and submitted to different ripening conditions (temperature / time). Influence of ripening time, culture concentration and ripening temperature on final concentrate-composition was tested. The most adequate cheese-model (concentrate) was evaluated in laboratory scale applying 6 different mixed cultures of wild strains of mesophilic lactic acid bacteria (*Lactococcus lactis* subsp. *lactis; Lactobacillus brevis, Leuconostoc mesenteroides; Lactobacillus plantarum*). With the same mixed cultures 6 pilot scale and 3 industrial scale semi-hard cheeses were manufactured and ripened during 3 weeks in parallel. All cheeses were analyzed chemically and sensorially, and compared to the concentrates.

Initial culture concentration did not influence final concentrate-composition. At lower ripening time (7 days) lactic acid content still increased due to a continuous degradation of lactose. Ripening temperature and proteolysis were correlated: increasing temperature caused higher non-protein nitrogen content. However, temperature seemed not to influence lactic acid and acetic acid concentration of the slurry.

The tested mixtures of cultures showed different behaviour in concentrate and in cheeses (citrate degradation, lactic acid fermentation). This may be due to varying growth of single strains of the mixture in different media and the longer ripening time of cheeses. Differences in chemical composition were only partially revealed by sensory evaluation.

Keywords: cheese ripening, mesophilic bacteria, cheese model

P116 ENVIRONMENTAL CONDITIONS INFLUENCING GROWTH AND CONIDIOGENESIS OF *PENICILLIUM CAMEMBERTI* ON CHEESE MODELS

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Radial growth and conidiogenesis of four technological different strains of *Penicillium camemberti* were investigated in a fractional factorial design, covering conditions relevant for white mould cheese production. The following factors were investigated in combinations: $CaCl_2$ (0 and 0.01%); Lactose (0 and 3 %); Glucose (0 and 0.5 %); Galaktose (0 and 0.5 %); pH (4.7 and 5.2); NaCl (0 and 4 %); Citrate (0 and 0.2 %); Lactate (0 and 3 %) and mineral solution (0 and 0.1 %).

The loadings plot below from the Partial Least Squares Regression analysis (APLSR) of the collected data gives an overview of the results. Radial growth (dia) by all investigated *Penicillium camemberti* isolates were suppressed by 4 % NaCl (situated opposite of each other in the plot). Lactose (3%) suppressed conidiogenesis (spo) in all isolates, whereas high pH (5.2) promoted conidiogenesis (spo). Other carbohydrates had varying effects on conidiogenesis.

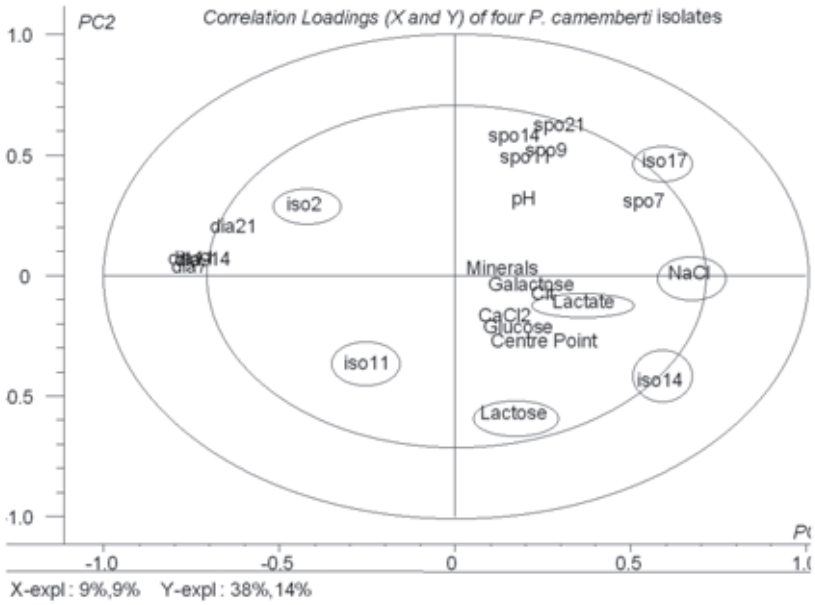
Some factors only affected individual isolates: pH promoted conidiogenesis in isolate 2; lactate suppressed growth and promoted conidiogenesis in isolate 11 whereas it suppressed both growth and conidiogenesis in isolate 17.

Citrate (0.2 %) and $CaCl_2$ (0.01%) suppressed growth and conidiogenesis of isolate 11. NaCl promoted conidiogenesis and high pH suppressed growth of isolate 17.

Growth and conidiogenesis of the individual isolates can also been seen from the figure. Isolate 2 grew relatively fast and had a relatively large conidiogenesis. Isolate 11 grew fast but had relatively low conidiogenesis. Isolate 14 grew relatively slow and had a low conidiogenesis. Isolate 17 grew relatively slow but had a relative high conidiogenesis.

The result can serve as guide for which factors in the production of white mould cheese that is important for a given strain of P. camemberti and by this facilitate the choice of optimal concentrations of factors for longer shell-life and control of conidiogenesis.

Keywords: *Penicillium camemberti,* ecophysiology, conidiogenesis, radial growth.



P117 PHYSIOLOGICALLY CHARACTERIZED YEASTS ISOLATED FROM RAW MILKS AND RAW MILK CHEESES

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Countries of the Mediterranean area are characterized by production of artisanal cheeses, obtained from goat, sheep, cow and buffalo raw milk. The numbers and species of yeasts in the different cheeses are variable, but some species are more frequently detected than others. Despite the frequent occurrences of yeasts in many dairy products, it is not generally accepted that these yeasts contribute significantly to the quality of the final product.

In the present work, 205 strains of Calabrian yeasts isolated from four samples of goats' milk, six samples of ewes' milk, 22 samples of goats' cheese made from raw milk (Caprino d'Aspromonte) and nine samples of ewes' cheese made from raw milk (Pecorino del Poro) were physiologically characterized.

In order to evaluate the physiological biodiversity of the dairy yeasts, the occurrence of some properties, such as the fermentation of glucose, galactose, and lactose, the assimilation of lactic acid and citric acid, the ability to grow at different concentrations of salt (5-10-15% NaCl), and the H_2S production on BiGGY agar was examined.

On the basis of these tests, only one yeast with identical characteristics and isolated from the same sample was maintained, the other strains being excluded: thus the number of dairy yeasts was reduced to 74.

These isolates were further studied for their proteolytic and lipolytic activity, and for their behaviour in the presence of nine autochthonous lactic acid bacteria (six coccal-shaped and three rod-shaped LAB) using the spot-on-lawn assay in Petri plates.

The potential implications of the results for further selection of the best strains as starter cultures for cheesemaking are discussed.

Keywords: dairy yeasts, physiological characterisation, yeast/lactic acid bacteria interaction

P118 DEVELOPMENT OF PERMEABILISED *LACTOCOCCAL* CELLS FOR APPLICATIONS INVOLVING THE HYDROLYSIS OF CASEIN DERIVED HYDROPHOBIC PEPTIDES

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A system has been developed optimising a *Lactococcal* strain for the purpose of de-bittering of hydrophobic peptides. The work involved a combination of approaches, conventional strain development coupled with use of food grade chemical agents to permeabilise the intact cells.

A traditional dairy *Lactococcal* strain was selected which, in a soluble production media, exhibited both good growth and high aminopeptidase (pep) N activity. The targeted culture was then treated with UV light and further screened to obtain a final strain possessing three times the pepN activity of the parent strain (pep XP activity remained constant).

Cell cream containing this strain was then dosed with varying levels of Sodium-lauryl-Sulphate in order to increase the detectable pepN activity with out corresponding cell lysis. The resultant material was shown, through sensory analysis and HPLC, to be useful for de-bittering casein hydrolysates.

Keywords: Permeabilised *Lactococcal* cells, de-bittering, hydrophobic peptides

P119 CORRELATION OF PEPTIDASE ENZYME ACTIVITY IN *LACTOBACILLUS HELVETICUS* DPC 4571 WITH PEPTIDASE GENE HOMOLOGUES IDENTIFIED FROM SEQUENCING

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Cheese manufactured using *Lactobacillus helveticus* DPC 4571 as a starter adjunct has previously been shown to have commercially interesting flavor characteristics. Levels of peptides and free amino acids were significantly increased in these cheeses, suggesting that this strain has a potent peptideolytic system. This study reports on a combined biochemical and genomic approach to elucidate the peptideolytic system of DPC 4571. The genome of *Lb. helveticus* DPC 4571 is currently being sequenced. The sequence data, generated to date, was imported into the Kodon software package and the FASTA search engine was used to search for peptidase gene homologues. Twenty-four potential peptidases were identified based on amino acid sequence homology, including multiple dipeptidases and endopeptidases. In order to determine if these peptidase activities were present and biologically active, a cell-free extract (CFE) of DPC 4571 was obtained from growth of the strain to mid-exponential phase in milk. This CFE was fractionated on a Mono Q column. To aid identification of specific peptidase enzymes the fractions were assayed for activity against various substrates including Leu-AMC, Lys-AMC, Pro-AMC, SAAP-AMC, Leu-Leu, Pro-Leu and Leu-Pro in the presence or absence of specific peptidases inhibitors. The data obtained indicated that the majority of the aminopeptidase activity against the substrates used in the study was due to aminopeptidase N (PepN), although activity of aminopeptidase C (PepC) was also evident. A previously uncharacterized aminopeptidase activity was also detected. PepD activity against dipeptidase was also demonstrated, although PepV activity was not. Activity of proline specific peptidases PepQ, PepI and PepR were confirmed. The activity of endo-oligo-peptidases PepO and PepF was detected. Overall, there was a good correlation between the putative genes identified in the genome and the types and numbers of activities detected biochemically.

Keywords: peptidase, biochemical, genomic.

P120 EFFECT OF STARTER CULTURES ON LIPOLYSIS AND FLAVOR DEVELOPMENT OF GRAVIERA KRITIS CHEESE DURING RIPENING

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The influence of different starter cultures on lipolysis and the volatile compounds evolution of Graviera Kritis cheese during ripening was investigated.

Three batches of Graviera cheese were manufactured using mixtures of *Streptococcus thermophilus, Lactobacillus helveticus* (1:1) and *Propionobacterium freudreichii* subsp. *shermanii (*cheese A); *Lactococcus lactis* subsp. *lactis, L. lactis* subsp *cremoris, Streptococcus thermophilus, Lactobacillus helveticus* (1:1:10:2) and *Propionobacterium freudreichii* subsp. *shermanii (*cheese B); and *Lactococcus lactis* subsp. *lactis, L. lactis* subsp *cremoris, Streptococcus thermophilus, Lactobacillus helveticus (*2.5:2.5:1:1) *and Propionobacterium freudreichii* subsp. *shermanii* (cheese C) and compared with cheese D manufactured without starter cultures.

Fat hydrolysis was estimated by determination of the acid degree value (A.D.V.), and free fatty acids (FFA) were determined by gas chromatography. The volatile fraction of cheese was analyzed by head-space gas chromatography. The results sowed that the use of starter cultures for the production of Graviera Kritis seemed to affect in both ADV values and FFA concentration. ADV values significanty ($P < 0.05$) increased during ripening. This is also confirmed by the increase of FFA concentration during ripening. A high correlation was found between ADV and FFA.

Sixteen compounds include acetaldehyde, acetone, butanone, diacetyl, acetoin, alcohols and esters were identified and quantitatively determined. The identified compounds were similar for the four cheeses, although several quantitative differences were observed between the cheeses made with and without starter cultures, mainly in matured (90 days) cheese. The content of same flavour constituents as ethanol, 2-butanone, propyl alcohol, isobutyl alcohol and ethyl acetate was found to be higher in matured cheese D, than the other cheeses A,B,C.

Aroma compounds and FFA profile of Graviera Kritis seems to be affected not only by the addition of starter culture but also by the kind and the ratio of lactic acid stains used in cheese manufacture.

Keywords: Graviera Kritis cheese, aroma compounds, lipolysis, head space gas chromatography

P121 THE EFFECT OF AUTOLYTIC STARTER SYSTEMS ON FREE AMINO ACIDS, FREE FATTY ACIDS, VOLATILE COMPOUNDS AND SENSORY PROFILES IN CHEDDAR CHEESE

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Autolysis of lactic acid bacteria in the cheese matrix is considered to accelerate cheese ripening. The objective of this work was to investigate how starter systems which varied with respect to their autolytic potential influenced flavour components during ripening. Cheddar cheese was manufactured using three related starter systems which varied with respect to their autolytic properties. Starter system A (control) contained a blend of two *Lactococcus lactis* strains (223 and 227) which had a low level of autolysis. System B was identical to A but included an adjunct of a highly autolytic strain of *Lactobacillus helveticus* (DPC4571). System C consisted only of strain DPC4571 as starter. The cheeses were evaluated at 2, 6 and 8 months of ripening for individual free amino acids (FAA), free fatty acids (FFA), volatile compounds and sensory attributes. Levels of all FAA were highly elevated in cheeses B and C relative to A. By 8 months of ripening the main FAA were glutamate, leucine, lysine, serine, proline and valine. Levels of C6:0, C8:0, C12:0 and C18:0 FFA did not vary greatly over ripening, while levels of C10:0, C14:0, C16:0 and C18:1 were elevated in cheeses B and C. Principal component analysis of volatiles compounds separated cheese A from B and C. Cheeses B and C were associated with dimethyl disulphide, carbon sulphide, heptanal, dimethyl sulphide, ethyl butanoate, 2-butanone, and 2-methyl butanal and were grouped with notes of 'caramel' odour and 'sweet', 'acidic' and 'musty' flavour. Cheese A was associated with 2-butanol, 2-pentanone, 2-heptanone, 1-hexanol and heptanal and grouped with 'sweaty/ sour' odour and 'soapy', 'bitter' and 'mouldy' flavour. The results highlight the effect of autolytic starter systems on flavour components over the ripening period.

Keywords: Autolysis, volatile analysis, lipolysis, acceleration, flavour improvement.

P122 LIPOLYSIS AND PROTEOLYSIS IN CHEDDAR CHEESE MADE WITH STARTERS OF DIFFERING AUTOLYTIC PROPERTIES

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The aim of this study was to assess the patterns of proteolysis and lipolysis in Cheddar cheese made from starter bacteria differing in their autolytic properties; *Lactococcus. lactis* AM2 and *Lactobacillus. heliveticus* DPC4571 (fast lysing), and *L. lactis* HP, *L. lactis* 303 (slow lysing). Cheeses were manufactured in triplicate using a single strain of each starter, ripened at 8°C and sampled periodically over 224 days. The following analyses were carried out: compositional analysis after 14 days, microbiology, proteolysis and lipolysis during ripening. Autolysis of starters up to day 56 of ripening was monitored by determination of lactate dehydrogenase activity in expressed cheese juice.

Compositionally, all cheeses were within national recommendations set for Cheddar cheese. Starter viability and autolysis in cheese decreased in the order: AM2 > 4571 > HP > 303. Populations of non-starter lactic acid bacteria (NSLAB) were comparable in AM2, HP and 303 cheeses, but significantly lower in 4571 cheeses particularly in the early stages of ripening. Primary and secondary proteolysis increased in the order of 303 > HP > AM2 > 4571. Significant starter-related differences were evident in the levels of individual free amino acids formed in cheeses, and were particularly pronounced in 4571 and AM2 cheeses. Lipolysis, increased throughout ripening in all cheeses and was highest in cheeses made with 4571, while AM2, 303 and HP cheeses had comparable levels. The ratio of short ($C_{4:0}$ to $C_{8:0}$) and medium ($C_{10:0}$ to $C_{14:0}$) chain FFA increased relative to long ($C_{16:0}$ to $C_{18:1}$) chain FFA during ripening in all cheeses, indicating higher esterase activity than lipase activity. Both of the fast lysing strains significantly influenced proteolysis, but 4571 appeared to influence both proteolysis and lipolysis. This study highlights lipolysis as a dynamic process in Cheddar cheese, which may be starter-related.

Keywords: Cheddar, starter culture, autolysis, lipolysis, proteolysis

P123 LYSIS OF MESOPHILIC AND THERMOPHILIC STARTER STRAINS IN UF CHEESES

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In cheeses made from ultrafiltration retentate it has been shown that lysis of strain *Lactococcus lactis* AM2 and several mesophilic commercial starter strains is completely inhibited. Proteolysis and the general extent of ripening have also been shown to be far slower compared to traditional cheeses. The absence of starter lysis has been cited as a plausible explanation. The objective of this work was to confirm that the observed absence of lysis occurs for several known autolytic and non-autolytic lactococcal starter strains and also to elucidate whether lysis of thermophilic strains of *Lactobacillus helveticus*, *L. delbrueckii* subsp. *lactis* and *St. thermophilus* are influenced in the same way. Cheeses were made from UF retentate (FCV = 6) of microfiltered milk (0.8mm) using the following starter systems: (1) single culture of autolytic strains of *Lc. lactis* (AM2, US3), (2) single culture of non-autolytic strains of *Lc. lactis* (AM2-C, Z-144), (3) a mixed culture of of *Lc. lactis* Z-144 and *Lb. helveticus* LH1, (4) a mixed culture of *Lb. helveticus* (LH1, LH32, LH303) with *St. thermophilus* Z-1358 and (5) a mixed culture of *Lb. delbrueckii* subsp. *lactis* (LL14, LL51) with *St. thermophilus* Z-1358. Cell viability was followed over a 4 week ripening period by enumerating on selective media, lysis was monitored by measurement of LDH, an intracellular marker enzyme and also by immunodetection of intracellular proteins with species specific antibodies (PepX, D-LDH). For cheeses made with strains of mesophilic lactococci, regardless of the strain used, absence of cell lysis was confirmed in the first few weeks of ripening. For cheeses made using thermophilic starter systems, lysis was detected in the first weeks of ripening, especially for strain *Lb. helveticus* LH1.

Keywords: Lysis, lactic acid bacteria, UF cheese

P124 ENHANCEMENT OF RACLETTE CHEESE FLAVOUR BY ADDITION OF *PROPIONIBACTERIUM FREUDENREICHII* AS ADJUNCT CULTURE

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Adjunct cultures are selected strains of cheese related micro-organisms added to cheese milk to improve flavour development. The aim of this study was to investigate the ability of propionic acid bacteria (PAB), the main secondary flora found in Swiss type cheeses, to enhance the flavour of Raclette cheese, a semi-hard cheese.

Raclette cheeses were manufactured from pasteurised milk at an industrial plant with or without (control) PAB as an adjunct culture. A mixture of four *Propionibacterium freudenreichii* strains were used and cheeses were ripened for 12 weeks at 11 °C. The flavour of cheeses was assessed by a trained sensory panel and levels of free amino acids, free fatty acids and volatile compounds determined.

Populations of PAB were 9×10^6 cfu/g cheese at 3 days and grew to 2.3×10^8 cfu/g cheese at 12 weeks. The contaminating PAB remained under 3×10^3 cfu/g in control cheese. Eye formation was not observed in cheese containing PAB which would have been considered a defect. PAB fermented lactate to acetate and propionate, as expected, and produced also fatty acids by lipolysis, branched-chain volatile compounds (derived from isoleucine and leucine catabolism) and some esters. The biochemical changes induced by PAB in Raclette cheese were close to those previously reported in Swiss type cheese. Cheese containing PAB received significantly higher scores of odour and flavour intensity than the control cheese and was characterised by a 'propionic' and 'whey' odour and a 'sweet' taste.

PAB can therefore be considered as potential adjunct cultures to enhance or modify cheese flavour development.

Keywords: *Propionibacterium*, flavour, Raclette cheese, volatile compounds.

P125 DNA EXTRACTION FROM THE CHEESE AND MICROBIAL ECOLOGY ASSESSMENT BY MOLECULAR TOOLS, IN PARTICULAR TTGE

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Molecular characterization of bacterial ecosystems has been used increasingly in the field of food microbiology. Indeed, diversity cannot be described solely by morphological and physiological features, which can be common to bacteria from phylogenetically distant taxa. By contrast, closely related genera may show a rich morphological and physiological diversity. It has been reported that conventional methods, involving a culture step, reveal only a small fraction of the microorganisms, the one able to grow.

Investigation of fermented food ecosystems follow now a polyphasic approach, including phenotypic, physiologic and molecular characterization. A recent tool within this polyphasic strategy corresponds to the highly specific, partial analysis of 16S rDNA sequences by temperature gradient gel electrophoresis (TGGE), or the close method, denaturing gradient gel electrophoresis (DGGE). However such methodology requires a DNA extraction step which should be as representative of the microbial ecosystems as possible.

Here, we report a combination of a new DNA extraction method from dairy products with a sensitive TGGE analysis of lactic acid bacteria**.** The DNA purification does not include any phenol/chloroform step but spin columns. Recovery of DNA was evaluated by fluorimetry quantification, showing that bacterial numeration and DNA quantification are well correlated, allowing a representative molecular analysis by TTGE. The detection limit of the method has been evaluated : minoritary bacterial species are detected if they represent more than 0,1% of the most dominant species. A marker corresponding to the main lactic acid bacteria species encountered in dairy product has been defined. Several commercial products were analysis according to this methodology, in three independent experiments : i) cheeses as raw milk Camembert, pasteurized milk Camembert, Roquefort, Raclette, La Vache qui rit, Reblochon Fermier, Reblochon Laitier, Leerdammer, Tomme de Savoie, Comté, Livarot, fresh goat cheese, Beaufort, Büche de chèvre, Saint-Nectaire, Raw milk Brie, ii) probiotic fermented milk, iii) milk powders. The profiles were compared by using UPGMA algorithm combined with a Pearson correlation coefficient, in order to check the repeatability of the fingerprint technique. The homology between the tree profiles for each dairy product were higher than 80%. The amplicons which were not correlated with a band of the marker were cloned and sequenced to be identified.

In conclusion TGGE analysis shows a high repeatability, and can be a valuable tool in a polyphasic investigation of microbial ecology of dairy products.

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Keywords: DNA extraction,TGGE, repeatability, ecosystems.

P126 INTERACTIONS BETWEEN CHEESE-RIPENING MICRO-ORGANISMS IN RELATION WITH VOLATILE FLAVOUR COMPOUNDS SYNTHESIS

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Cheese curd can be viewed as a complex biochemical habitat in which a variety of micro-organisms coexist, anabolizing substances and catabolizing others in order to live and grow. When metabolising curd constituents, micro-organisms establish different types of interactions. However, the precise contribution of each micro-organism to the cheese flavour development is still partly unclear.

Indeed, cheese-ripening micro-organisms have been previously tested for their ability to produce desirable aroma compounds in pure cultures either in chemically defined media (Spinnler at al., 2001; Bonnarme et al., 2000) or in cheese-like media (Berger, 1999). These approaches provided valuable knowledge of the pathways leading to the biogenesis of aroma compounds by cheese-ripening micro-organisms. However, many observations suggested that aroma production during ripening could result from multilevel interactions between micro-organisms.

The cheese-ripening yeasts *Kluyveromyces lactis*, *Geotrichum candidum* and *Debaryomyces hansenii* were grown on a cheese-curd medium either individually or in association with the cheese-ripening bacterium *Brevibacterium linens*. Various aspects of the interaction between the yeast and *B. linens* were investigated. Alcohols, aldehydes and esters were produced by yeasts *K. lactis* and *D. hansenii* at early stages of culture while volatile sulfur compounds (VSCs) were produced late during the ripening process where *B. linens* develops. VSCs production was highly improved in terms of variety and quantity by the association of the yeasts *D. hansenii* or *K. lactis* with *B. linens*. Lactic acid bacteria (LAB) were also associated with *B. linens* and one yeast. The effect of LAB on microbial interactions is evaluated.

This presentation gives evidence of metabolic complementarities between cheese-ripening micro-organisms for flavour compounds synthesis. This particular point is discussed.

Keywords: cheese ripening, microbial associations, aroma compounds.

P127 CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF MADDAFARA CHEESE, A TRADITIONAL SUDANESE CHEESE

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Maddafara cheese was made from pasteurized goat's milk, a mixture of goat and cow's milk and cow's milk. The raw milk and the Maddafara cheese were chemically and microbiologically analyzed. The chemical composition of the raw goat and cow's milk differed not significantly in most of the parameters. The moisture, ash, titrable acidity, and pH were higher while the fat, total protein, total solids contents and the yield were lower in goat's milk as compared to cow's milk. The samples of raw goat's milk showed viable counts of $9x10^5$ cfu/ml of mesophilic aerobes, $45x10^3$ cfu/ml of *Bacillus cereus,* 2.1×10^3 cfu/ml of coliforms and an inherent lactic acid bacterial count of $5.6x10^3$. The high population of the different microbial groups indicated poor hygienic conditions of milk handling. The chemical composition of the cheeses showed that the Maddafara cheese made from goat's milk was rich in total protein (28.52 %), moisture (34.8 %) titrable acidity (0.93% lactic acid) and the product had a pH of 5.45. The cheese from pure goat milk had low yield (10.4 kg/100 kg milk) as compared to the two other cheeses. The microbial counts in *Maddafara* cheeses varied significantly in the different samples. Those results suggested that it was possible to use goat's milk or a mixture of goat and cow's milks for making Maddafara cheese of satisfactory hyiegenic quality.

Keywords: Maddafara cheese, goat's milk, chemical composition, microbiological analyses

P128 PREPARATION OF A LACTIC STARTER FOR THE MANUFACTURE OF AN ALGERIAN TRADITIONAL CHEESE

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In Algeria, since centuries, a traditional fresh cheese called Djben is manufactured in farmhouses of certain areas of the East of the country, by coagulation of raw ewe's milk with the wild cardoon flower (*Cynara cardunculus L*).

The aim of the present paper is to study the mesophilic bacteria lactic flora of this cheese in order to make a lactic starter with the principal strains.

Lactococcus was the main genus. Three strains belonging to *Lactococcus lactis* were associated into lactic starter proposed for manufacture of the same traditional cheese but with a pasteurized milk. The objective is to avoid the risks of potential contaminations of the raw milk at the same time as to preserve the sensorial properties of the cheese due to these bacteria. The cheese obtained presented rheological and organoleptic properties similar to those of the cheese made with raw milk.

Keywords: lactic starter, traditional cheese, algeria

P129 THE ANTIMICROBIAL ACTIVITY OF NISIN-PRODUCING STRAIN *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* LTM 32 AGAINST *BACILLUS CEREUS* DMF 2008 IN SOFT FRESH CHEESE

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Some wild bacteriocin-producing strains of lactic acid bacteria, known for their protective action, could be used individually or in combination with industrial strains in dairy production.

This work deals with the basic genotypical characterization (the localization of *NisA* and *NisB* genes of the nisin biosynthesis gene cluster) of bacteriocin-producing wild strain *Lactococcus lactis* subsp. *lactis* LTM 32, isolated from Vietnamese fermented milk, proving that its bacteriocin is nisin. Further the antimicrobial activity of this type of nisin against *Bacillus cereus* DMF 2008, directly added at the concentration of 10^7 cfu/g into soft fresh cheese prepared in laboratory conditions, was evaluated.

Cheese was stored for 26 days at 4 °C and 25 °C and the killing effectiveness of nisin-producing strain *Lactococcus lactis* subsp. *lactis* LTM 32 against *Bacillus cereus* DMF 2008 was estimated. *Lactococcus lactis* subsp. *lactis* LTM 32 producing nisin at the initial concentration of 4.0 mg/kg of soft fresh cheese caused growth reduction in *Bacillus cereus* DMF 2008 by 6 log cycles and by 5 log cycles during 26 days of storage at 4 °C and at 25 °C. Nisin-non-producing commercial mesophilic starter culture CH 01 possessing nearly the same acidification ability was used at comparable cell concentration as nisin-producing strain and showed considerably less suppression of *Bacillus cereus* DMF 2008 during the same storage conditions.

Keywords: growth reduction, *Bacillus cereus*, nisin, *Lactococcus lactis*

P130 CHARACTERIZATION OF THE PIGMENTED MICROFLORA ISOLATED FROM SOME „PROTECTED DESIGNATION OF ORIGIN“ RED-SMEAR SOFT CHEESES PRODUCED IN FRANCE

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Red-smear ripened soft cheeses are popular dairy products in Europe. Among these are French cheeses such as Maroilles, Livarot, Epoisses…, German varieties (e.g., Tilsit, Limburger…), Italian (Taleggio) and Belgium (Herve) cheeses. Most of them are protected by the European Union through the PDO (Protected Designation of Origin) system which covers the term used to describe foodstuffs which are produced, processed and prepared in a given geographical area using recognised know-how. The red smear of these cheeses originates from the synthesis of pigments by bacteria. Research efforts have to be conducted to improve our knowledge on the pigmented microflora. The work at our laboratory focused on : a) objective measurements of the colour of various cheese rinds (Lab colorimetric system), b) isolation and characterisation of the pigmented bacteria, c) spectrocolorimetric measurements on single strains, d) HPLC analysis of pigmented extracts, e) chemical synthesis of the microbial pigments. Some of these points (HPLC analysis of microbial pigments, intensive use of Lab colorimetric system) are innovative compared to works published in recent years. In fact, the bacterial genera and species active in this dairy process are not yet really known and described for all the European red-smear soft cheeses. The action of *Brevibacterium linens,* which is the only species sold for colour development of cheese rind, is still under discussion in numerous papers. Other genera such as *Arthrobacter*, *Corynebacterium*, *Kocuria*, *Microbacterium* and *Micrococcus* were found in our experiments as in similar previous studies and may have an impact. Moreover, in our experimental work, each PDO cheese seems to have specific ratios of unpigmented / yellow / orange strains.

Keywords: cheese, ripening, pigment, microflora

This work was supported by ACTIA (Association de coordination technique pour l'industrie agro-alimentaire) research programme 99.14, the French Minister of National Education, Research and Technology (M. E. N. R. T.), Degussa France and four cheese producers.

P131 ECOLOGY OF LACTOBACILLI DURING PRODUCTION OF AN TRADITIONAL CAMEMBERT CHEESE AS EVALUATED BY PCR-TGGE

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Traditional Camembert cheese is a soft ripened cheese made from raw milk in the Normandy area in France. In registered designation of origin (RDO) Camembert, most or all lactobacilli are NSLAB (non starter lactic acid bacteria), originating from farm environment, cows and dairy manufacture environment. The *Lactobacillus* genus is the largest genus within the LAB group, with 92 species and 15 subspecies described to date (www.bacterio.cict.fr, 27.06.03). It has been the object of many taxonomic changes both within and between genera. Currently, we have a fragmentary view of the diversity of the lactobacilli population within the milk and cheese during its transformation.

The aim of this study was to describe, using mostly PCR-TGGE (temperature gradient gel electrophoresis), the biodiversity in lactobacilli populations and their evolution during cheese making in three factories at two seasons (autumn when cows were housed and spring when cows were grazing). The diversity was investigated by a combination of culture-independent approach by PCR-TGGE and sequencing and culture dependent approach; including phenotypic characterization, PCR-TGGE and pulsed field gel electrophoresis (PFGE).

In the three factories, TGGE patterns of total DNA from samples taken at five stages from ripened milk to 60-days old camembert cheese, revealed two species as dominant from the 14th day of ripening, depending on dairies. Identification of *Lactobacillus* isolates by phenotypic characterization and PCR-TGGE confirmed the presence of the same dominant species but also detected other patterns. PFGE analysis of isolates from one dominant species indicated a large diversity of strains throughout the cheese-making procedure and in the three factories.

PCR-TGGE analysis on total DNA combined with molecular analysis of isolates can effectively describe one part of the complex diversity of the lactobacilli population throughout the Camembert cheese-making procedure, giving useful information for preservation of artisanal food technology.

Keywords: PCR-TGGE, *Lactobacillus*, Camembert cheese, biodiversity.

P132 THE LUMP CHEESE FROM RAW SHEEP MILK: PRE-BRYNDZA CHEESE

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The fact that lump cheese from sheep milk, so called pre-Bryndza cheese is made from raw sheep milk at the upland cottages (salashes) brings certain health risk. *Staphylococcus aureus* belongs to the most frequent pathogens in sheep milk that is milked and processed at farm conditions.

The results of eight field examined trial productions of lump cheese at farm level in the period of July are summarily presented in figure referring to dynamics of pH-values measured inside the curd during its processing into the young lump cheese within 1st to 4th d of production. At the beginning, after adding the rennet into milk, the pH-value was 6.8 and temperature from 30 to 32 °C. Curd acidifying started between 10 and 20 hours and went on intensively till 40th h. After finishing the second day of fermentation, the required level of acidity, pH = 4.9 to 5.2 was reached.

Within our field trials, the initial numbers of *S. aureus* in sheep milk were 170 cfu/ml, in cheese 1.5 $\times 10^6$ cfu/g after 3 d (at optimal acidity of the cheese pH = 4.8) and 500 cfu/g after 6 d (at pH 4.9).

Overall requirement of STN 1138 and EU Directive 92/46 concerning the level of *S. aureus* in lump cheese made from raw sheep milk (m = 1000 and M = 10 000 cfu/g; c = 2) was not seemed quite realistic. From the point of these official requirements, the time of sampling and examination of *S. aureus* in lump cheese made from raw milk is not clear.

Keywords: sheep Lump cheese, fermentation at farm level, *S. aureus*

P133 EFFECT OF *LACTOBACILLUS RHAMNOSUS* VT1 AND TEMPERATURE ON GROWTH OF YEAST *CANDIDA MALTOSA* YP1

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The growth dynamics of oxidative, imperfect yeast *Candida maltosa* YP1 in ultrapasteurized milk innoculated with 18 h culture of *Lactobacillus rhamnosus* VT1 (1.0; 2.5; 5.0; 10 and 15 % v/v) at 10, 17 and 21 °C was investigated. Strain *C. maltosa* YP1 was isolated from the surface of spoiled fruit yoghurts in Slovakia probable for the first time (Lauková et al., 2002).

Addition of *Lb. rhamnosus* VT1 in milk resulted in partially inhibition of *C. maltosa* YP1 growth at all tested temperatures, especially at 21 °C. The initial *Lb. rhamnosus* VT1 concentrations influenced much more lag-phase duration in comparison with decreases of growth rate of *C. maltosa* YP1. The influences of *Lb. rhamnosus* VT1 density on *C. maltosa* YP1 growth rate were at 10, 17 and 2 °C linearised and statistical considerable ($R^2_{(\mu\ 10\ °C)} = 0.9615$; $R^2_{(\mu\ 17\ °C)} = 0.9833$; $R^2_{(\mu\ 21\ °C)} = 0.7824$). Decreases of natural logarithm of lag-phase prolongation (ln λ) as dependence of increasing *Lb. rhamnosus* VT1 concentration were at 17 and 21°C described using the polynomic equations and at temperature of 10 °C the lag-phase duration was modelled according to linear equation ($R^2_{(\lambda\ 10\ °C)} = 0.9732$; $R^2_{(\lambda\ 17\ °C)} = 0.9998$; $R^2_{(\lambda\ 21\ °C)}=0.9779$).

The influence of 10 % inoculation by culture of *Lb. rhamnosus* VT1 in UHT milk at 17°C was multiple significant in comparison with the influence of lactic acid (0.27 %) at 16 °C. Lag-phase duration and generation time of *C. maltosa* YP1 were in UHT milk innoculated with 18 h. culture of *Lactobacillus rhamnosus* VT1 (10 % v/v) 6.5 times or 2.5 times prolonged in comparison with growth parameters of *C. maltosa* YP1 observed in model glucose solution with yeast extract and lactic acid added in concentration of 0.27 % (Valík, Görner and Lauková, 2003).

Keywords: *Candida maltosa* YP1, mathematical modelling of microbial growth, predictive microbiology

P134 THE EFFECT OF NATAMYCIN ON THE SHELF LIFE OF UF FETA CHEESE

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Natamaycin as an antifungal agent was used to retard mold and yeast growth in concentrations: 0.5, 1, 2 and 4 mg/kg onto UF Feta cheese by surface spraying method in spring and summer. Cheese samples were stored at two different temperatures after reaching the optimum pH. One group in a refrigerator at 4 °C and the other in an incubator at 25 °C to accelerate fungi growth. pH, yeast & mold count, total microbial count and organoleptic properties consist of odour, taste, colour and acceptability were analyzed at 1, 14, 28, 56, 90, 120, 150 and 180 days for samples stored in the refrigerator and for the latter at 1, 10, 20, 30, 40, 50, 60 and 70 days after production. No significant differences could be found in the pH between samples treated with natamycin and control cheese, so natamycin has no adverse effect on the starter culture activity. The difference in fungi counts between treated and control cheeses was statistically significant for cheese containing 1, 2 and 4 mg/kg natamycin (at 95 or 99% levels depending to season and temperature of storage) so that shelf life was increased about 25–50 % in spring and 50–500 % in summer depending to storage conditions. Natamycin had no effect on the total microbial count in all treatments. Test panel evaluated organoleptic properties of samples and blanks as the same and there was statistically no significant difference between them, although in cheese containing 4 mg/kg natamycin panel stated smart pain on tongue nib in a few cases.

Keywords: natamycin, Feta cheese, shelf life

P135 THE INFLUENCE OF PACKAGING ON THE QUALITY OF LACTIC ACID CHEESE

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Application of the modified atmosphere in the packing of lactic acid cheese does not guarantee the inhibition of the development of all groups of micro organisms present in the product before storage.

The objective of the research was the assessment of changes of lactic acid cheese surface micro-flora depending on the system of packing and the time of products storage.

Considerable differences in the rate of change of micro-flora population depending on the applied system of packing have been found.

The research carried referred to the acidic lactic cheese packed in vacuum and non-vacuum conditions, as well as packing laminates applied for the cottage cheese, i.e. PA/PE and Cryovac type laminate.

In order to determine the number of micro organisms, the selective media of Merck and bioMerieux for mould and yeast, for enterococci, staphylococci, *Escherichia coli*, and for psychrotrophic bacteria.

The mathematical models describing the observed changes may be applied in practice solely for relatively anaerobic micro flora. The mathematical equations cannot be applied in describing of changes in the size of population of psychrotrophic bacteria and mould.

The linearised models of change may be applied solely for relatively anaerobic bacteria.

The change rate of the surface microflora is crucially related to the different conditions present inside of the packaging in both of the lactic acid cheese packing systems.

The received results indicate a significant influence of the space between the packaging and the surface of lactic acid cheese on the growth of population of the tested micro organisms.

Keywords: lactic acid cheese, microflora, system of packing, mathematical model

P136 SURFACE MICROFLORA OF TILSIT-CHEESE

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In 2002 approximately 13500 tons of Tilsit, a smear ripened semi hard cheese, were produced in Austria. As other smear cheeses, this variety is characterised by the succession of complex microbial communities on its surface. In several attempts the smear microflora, particularly the bacteria, of these cheeses, have been only poorly described because they are difficult to identify using classical phenotypic methods

In this study, we characterised surface microflora of Tilsit produced in 3 different plants at 3 stages during ripening. At each sampling point 20 apparently different yeasts and bacteria were isolated. Additionally 179 bacteria and 164 yeasts isolated previously from Tilsits of 15 dairies were included into the project.

Yeasts were dereplicated and identified by Fourier Transform Infrared (FTIR) spectroscopy and bacteria were dereplicated by REP-PCR. Representative bacterial isolates from the different clusters were identified by a polyphasic approach including traditional methods, FTIR-spectroscopy, PCR and 16S-RNA sequencing. The bacterial flora was additionally examined by direct extraction of DNA from cheese and denaturing gradient gel electrophoresis (DGGE)

Debaryomyces hansenii (with high intraspecies variability) dominated the yeast flora in all cheeses and at each ripening stage (> 80 %) and pellet forming *Galactomyces ssp*. were isolated from all products in the late ripening stage.

Biodiversity in the bacterial surface flora was much higher with 2 *Arthrobacter ssp*., 2 *Brachybacterium ssp*., 6 *Brevibacterium ssp*., 5 *Corynebacterium ssp*., 2 *Microbacterium spp*., 3 *Kocuria spp*. and 5 *Staphylococcus spp*. identified. In addition 2 presumably new taxa were isolated (description in progress).

Keywords: Tilsit, biodiversity, surface-microflora, cheese

P137 THE COLOUR OF *BREVIBACTERIUM LINENS* AND *ARTHROBACTER SPECIES* DEPENDS ON THE CHEESE-MAKING TECHNOLOGY

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The colour of smear cheeses is traditionally due to the bacterial flora. This study was carried out to evaluate the effects of curds on the colour of two *B. linens* (ATCC9175; C208, GMPA) and one *Arthrobacter* sp. (C99, GMPA). One day old curds of Munster, Reblochon, Livarot or Epoisses were used to prepare 60 % cheese media, which was deacidified with *Debaryomyces hansenii* (304, GMPA) to reach pH 5.8. After a thermal inactivation of the yeast and addition of agar-NaCl, each bacterium was inoculated on the medium surface and incubated at 12 °C for 28 days. Each bacterium was analyzed in triplicate independent trials. For each biofilm, colour was evaluated by L*C*h° (brightness, chroma, hue angle) spectrocolorimetry. Whatever the curds used, after deacidification and incubation, on day 28 the non seeding medium (reference) had the same clear yellow colour (C* 18, h 90°). The hue angle of *B. linens* ATCC9175 biofilms remained constant whatever the curds used (h=64 ± 1°, corresponding to orange). Their saturation also remained constant (C* 70,

corresponding to a very dark colour) and the differences observed for these colorimetric parameters were not statistically significant. The colour of *B. linens* C208 and *Arthrobacter* C99 biofilms obtained from Epoisses curd were not significantly different than the one of non-inoculated media. The biofilms of *B. linens* C208 obtained from Livarot or Munster curds presented the same colour (C* 24, h 85°). They were more yellow and darker than the ones obtained from Reblochon curd (light orange, C* 18, h 50°). For *Arthrobacter* C99, the same repartition of colour was observed than for C208. If *B. linens* ATCC9175 seemed to be able to make an adaptation to every curd when it was used in pure culture, it was not the case for the other strains tested. Their colour was strongly related to the curd technologies.

Keywords: smear cheeses, *B. linens*, *Arthrobacter*, L*C*h° spectrocolorimetry

P138 FREE FATTY ACID EVOLUTION IN CAMEMBERT CURD MEDIA SEEDED WITH *KLUYVEROMYCES LACTIS*, *GEOTRICHUM CANDIDUM* OR *PENICILLIUM CAMEMBERTI*

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Phenomena generating free fatty acids (FFA) and their derivatives (methyl ketones…) in cheese are a major aspect of ripening. One day old Camembert-type cheeses were used to prepare a 60 % cheese medium. Cheese-type media seeded with *Kluyveromyces lactis* (44_8, GMPA collection), *Geotrichum candidum* (D, Degussa) or *Penicillium camemberti* (R, Degussa) were sampled for FFA analytical purposes between day 4 and 28. Cheese media deacidified by *Candida lambica* (Z1, GMPA) until pH 6.0 was reached were also seeded with *Brevibacterium linens* (ATCC9175). Three incubation replicates performed under 12°C, 95 % of relative humidity and atmosphere controlled conditions showed similar ripening characteristics. The FFA were extracted with diethyl ether and methylated with TMAH, separated and quantified by direct gas chromatography and identified by mass spectrometry. The concentrations of valeric, heptanoic, undecanoic, pentadecanoic and heptadecanoic acids were negligible whatever the time and they were used as internal standards. The degree of lipolysis was shown by the increase in concentration of FFAs during incubation. The concentration of butyric acid increased rapidly in the first period of ripening, reaching its maximum values on day 4 for media seeded with *K. lactis*, on day 7 for *G. candidum* and *P. camemberti*, and on day 15 for all the part of the cheese media. For *K. lactis* the most abundant FFAs were butyric and lauric acids, reaching their maximum on day 4 (620 mg/kg) and between day 7 and 14 (120 mg/kg), respectively. For cheese media seeded with *G. candidum* the most abundant ones were palmitic and stearic acids, reaching their maximum on day 21 (148 mg/kg) and on day 28 (310 mg/kg), respectively. For *P. camemberti*, the cheese media have shown the same major FFA as for *G. candidum* media, but they presented 2 maximums, a first one on day 14 and a second one on day 28.

Keywords: free fatty acid, surface microflora, Camembert-type curd

P139 PCR DETECTION OF BACTERIOPHAGES FROM DAIRY THERMOPHILIC LACTOBACILLI

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Phage attack has always been a major problem in industrial fermentation, especially in the dairy industry. However, some studies on thermophilic starters have also showed that lytic phages often coexist with bacterial strains, even if these starters were contaminated by high levels of phages.

In this work, 32 natural whey starters from Italian hard, long-ripened cheeses representative of 28 cheese plants located in five different regions in the north of Italy, were studied.

Virulent phages active against thermophilic lactobacilli were found in 16 of the 32 natural whey starters (21 from Grana Padano, 9 from Parmigiano Reggiano and 2 from Provolone cheese). Within the 513 lactobacilli isolated, 54 (15,1 %) out of 358 isolates of *Lactobacillus helveticus* and 22 (24,2 %) out of 91 isolates of *Lactobacillus delbrueckii* subsp. *lactis* showed lysis.

The presence of these phages was investigated using traditional techniques which, although being reliable, are also time-consuming. Therefore, we developed a rapid PCR method for detection and classification of *Lactobacillus delbrueckii* subsp. *lactis* bacteriophages isolated from natural whey starters.

A couple of primers were designed on the basis of conserved regions within the nucleotide sequence of the major tail protein gene. Using these primers, a distinct 400-bp amplification product was achieved for all of the 22 (100 %) phages assessed. The method showed also high specificity (absence of amplicons from other LAB phages).

Serial diluition of phages in whey was used to determine the sensitivity of the PCR assay. The method showed a detection limit of about 10^5–10^6 PFU/ml, which was improved to10^2–10^3 PFU/ml after a re-amplification of the PCR product.

The PCR approach confirmed to be a potentially useful tool to screen for the presence of phages from whey starters. The method was rapid, effective and easy to use.

Keywords: bacteriophages, thermophilic lactobacilli, dairy starter, cheese

P140 EFFECT OF COMBINATION OF HIGH PRESSURE TREATMENT AND BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA ON THE SURVIVAL OF *LISTERIA MONOCYTOGENES* IN RAW MILK CHEESE

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The combined effect of high pressure (HP) treatment and milk inoculation with bacteriocin-producing lactic acid bacteria (BP-LAB) on the survival of *Listeria monocytogenes* during ripening of a semi-hard cheese produced from raw cow milk was investigated.

Cheeses were manufactured from raw milk inoculated with *L. monocytogenes* at 4.80 log cfu/ml, a commercial starter culture and one of seven strains of BP-LAB, added as adjuncts at 0.1 %. A control cheese was made from milk not inoculated with BP-LAB. Cheeses were HP treated at 10 °C on days 2 or 50 for 10 min at 300 MPa or for 5 min at 500 MPa.

On day 3, *L. monocytogenes* counts were 7.03 log cfu/g in control cheese (no HP treatment). Milk inoculation with different BP-LAB lowered *L. monocytogenes* counts on day 3 with respect to control cheese by up to 0.97 log cfu/g, HP treatment on day 2 at 300 MPa by 0.90 log cfu/g and HP treatment on day 2 at 500 MPa by 5.02 log cfu/g. Combinations of BP-LAB with HP treatment on day 2 at 300 MPa lowered *L. monocytogenes* counts on day 3 by up to 3.20 log cfu/g and combinations of BP-LAB with HP treatment on day 2 at 500 MPa achieved total elimination of the pathogen on day 3.

On day 51, *L. monocytogenes* counts were 6.34 log cfu/g in control cheese (no HP treatment), and were lowered by up to 1.49 log cfu/g by BP-LAB. *L. monocytogenes* could not be detected on day 51 in 13 out of 16 cheeses HP-treated on day 50 and was recovered by enrichment of 1 g samples in the rest.

Some of the combinations of BP-LAB with HP treatments on day 2 showed a synergistic effect, a fact which permits the improvement of cheese microbiological quality with milder HP treatments while preventing the appearance of quality defects due to treatments at higher pressures.

Keywords: cheese, *Listeria monocytogenes*, bacteriocin, high pressure

P141 SURFACE MICROFLORA OF LIMBURGER CHEESE

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The surface microflora of smeared cheeses is a complex consortium consisting of yeasts, coryneform and other Gram-positive bacteria. However, the composition and the interactions between the different ripening organisms are not known in detail.

In this study the microflora of three batches of Limburger cheese produced by one dairy was investigated at an early, middle and late stage of ripening. From each of the nine cheese samples 50 yeasts and 50 bacteria were isolated which resulted in a total of 450 yeast isolates and 450 bacterial isolates.

Screening and identification of yeast isolates was done by Fourier Transform Infrared (FTIR) spectroscopy. To confirm the FTIR-results, representative yeast strains were investigated by physiological tests. Bacterial isolates were screened by rep-PCR. Representative bacterial strains were identified and characterized using a polyphasic approach including several phenotypic and genotypic tests. Furthermore, DNA was extracted directly from the cheese surface and Denaturing Gradient Gel Electrophoresis (DGGE) was then applied to determine the number of different bacterial taxa present and to see whether there were unculturable bacteria on the cheese surface.

The yeast flora solely consisted of *Debaryomyces hansenii* and *Galactomyces geotrichum*. *D. hansenii* dominated the flora during the different stages of ripening of all batches, except for the early stage of Batch 2. The larger part of the bacterial isolates was identified as belonging to the *Arthrobacter nicotianae* group. Additionally, *Brevibacterium aurantiacum*, a recently described species, was found. The species diversity on the surface of Limburger cheese was not as high as expected according to the literature.

Keywords: Limburger, biodiversity, surface microflora, cheese

P142 ANTIFUNGAL EFFECTIVENESS OF *LACTOBACILLUS RHAMNOSUS* VT1 IN MODEL CHEESE SYSTEM

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The contamination of cheese with undesirable moulds is a serious problem. *Penicillium* is one of the predominant genera in the fungal mycoflora of semi-hard cheese. Also species of geni *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp. were found in Dutch-type cheese.

The antifungal activity of *Lb. rhamnosus* VT1 against three mould strains was evaluated by modified milk agar plate method. Two sets of experiments were carried out. The first one contained 2.5 % NaCl (w/w) in the medium and simulated the Dutch-type cheese surface. The second one without NaCl was carried out in order to evaluate the inhibitory effect of *Lactobacillus* strain. Both experiments run at 14 °C for 40 days and at 30 °C, for 6 days. The incubation at 14 °C, for 40 days, represented the Dutch-type cheese ripening.

Lb. rhamnosus VT1 grown in milk environment at 30 and 14 °C was found to be inhibitory against *Penicillium* sp. (90 % of inhibition at both temperatures) and *Aspergillus* sp. (20 % and 70 % of inhibition, respectively). *Geotrichum* sp. was not affected by *Lb. rhamnosus* VT1 metabolites at 30 °C, while its growth was supported at 14 °C. The antifungal activity of *Lb. rhamnosus* VT1 was also tested under laboratory conditions simulating the Dutch-type cheese surface. *Lb. rhamnosus* VT1 in combination with NaCl (2.5 % w/w) was found to be inhibitory against all indicator mould strains. After 6 days of incubation at 30°C *Penicillium* sp., *Aspergillus* sp. and *Geotrichum* sp. were inhibited by 45, 38 and 10 %, respectively. After 40 days of incubation at 14°C *Penicillium* sp., *Aspergillus* sp. and *Geotrichum* sp. were inhibited by 70, 30 and 15 %, respectively. Presence of NaCl (2.5 % w/w) in the growth medium has no effect on the viability of *Lb. rhamnosus* VT1, however it probably alters production of antifungal compounds.

Keywords: *Lactobacillus rhamnosus* VT1, mould, inhibition, Dutch-type cheese

P143 INHIBITION OF CHEESE SPOILING MICROORGANISMS BY METABOLITES FROM LACTOBACILLI

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This work presents production of antimicrobial supernatants by strains of *Lactobacillus rhamnosus* VTl, *Lactobacillus rhamnosus* LC 705 and *Lactobacillus plantarum* ALC01 and their inhibitory effect on the originators of redoubtable defects of ripened hard cheeses:
– *Aureobasidium pullulans* isolated from black stains on the surface of hard steamed cheese
– a mixture of sporulating anaerobes from hard cheese (Emmental - Type) with late blowing defect, without detailed identification.
A stationary cultivation in MRS broth at 37 °C under anaerobic conditions was performed to calculate growth characteristics.

Supernatants were obtained by following procedure: 1 liter of MRS broth from the end of exponential growth phase was centrifuged (4800 g, 20 min), pH of the supernatant was adjusted to 6.5 with 1 M NaOH and passed through a membrane filter (0.22 μm). A smooth coagulum precipitated from the cell-free filtrate by ammonium sulfate (40% of saturation). Precipitate collected by centrifugation (12000 g, 10 min) was re-suspected in a minimum volume of phosphate buffer (pH 6.5). Portions of this solution were applied to chromatography columns comprising carboxymethyl cellulose, Sephadex G-50 or Amberlite XAD-16, respectively, and eluted with 1 M NaCl. Partially purified supernatants from the strains LC 705 and VTl showed inhibitory effect against the yeast *Aureobasidium pululans* by agar diffusion assay, but no inhibitory effect was observed in case of ALC01 supernatant. On the other side, the strains of LC 705 and ALC01 showed significant inhibitory effect of supernatant against sporulating anaerobes even before the purification on columns, while the strain VTI showed no inhibitory effect.

Keywords: antimicrobial metabolites, lactobacilli, cheese spoilage

P144 BIODIVERSITY OF LACTIC ACID BACTERIA PRESENT IN THE NATURAL MICROFLORA OF TRADITIONAL EWE'S RAW MILK FIORE SARDO CHEESE

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The inter-and intraspecies genetic diversity of lactic acid bacteria isolated from 24 h, 1- and 3-month-old home-made Fiore Sardo ewes' raw milk cheese was investigated. Cheese samples were collected from 2 farms located in different areas of Sardinia. A total number of

180 isolates (139 cocci and 41 lactobacilli) were taxonomically identified by using two molecular techniques, species-specific PCR and ARDRA. All the lactobacilli belonged to the facultatively heterofermentative lactobacilli group (FHL), mainly *Lactobacillus plantarum* and *Lactobacillus paracasei*. *Lactococcus lactis* and *Enterococcus* spp. were the only species found among cocci. This investigation showed that the development and the evolution during ripening of the different LAB species were different in the two cheeses. All the FHL and lactococci isolates were typed at strain level by means of plasmid profiling and AFLP technique. The study revealed that the FHL and lactococcal microbial populations of Fiore Sardo cheese were complex, not only 24h after manufacture, but also after 1 and 3 months of ripening. The genetic diversity at subspecies level, (n° of genotypes/n° of isolates) x 100, ranged from 80 to 100% during the three periods examined. The dominant genotypes at each sampling point were identified and the persistence of strains during cheese ripening shown. The use of two complementar molecular techniques allowed us to distinguish between closely related isolates and between isolates that probably belong to the same clonal lineage, better. The genetic complexity observed in the present study is of particular relevance in the preservation of the natural microflora of traditional Protected Designation of Origin (PDO) raw milk cheeses, as well as in the selection of new starter strains for the dairy industry.

Keywords: biodiversity, raw milk cheese, plasmid profiling, AFLP

P145 NEW APPROACH TO REBUILD SUBECOSYSTEMS OF LIVAROT CHEESES HAVING DESIRED AROMATIC AND TECHNOLOGICAL PROPERTIES

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The control of the ripening micro-flora, because of its role in a large number of functions in cheese (e.g.: color, flavor, texture,…), is a main goal for the cheese technologist.

Previous work has shown that quite often the flavor capabilities of a pure culture of bacteria are lost when cultivated in mixed culture. Moreover when mixed cultures of yeasts and bacteria were used only the flavor characteristics of the yeasts were retained showing the limitations of an additive approach of the microbial formulation of cheeses.

Here an alternative method of screening is proposed based on combinatorial chemistry principles. It starts directly from a complex ecosystem, adapted to the technological constraints and is simplified then by a selection of a subgroup of bacteria and yeasts able to produce the major ripening properties (mainly aromatic and desacidifying functions). They were assayed in cheese and compared to the one made with the initial mixture.

In the starting mixture, eighty-two different strains (33 yeasts and 49 bacteria), isolated from high quality Livarot were used. These were representative of the taxonomic diversity of the cheese (based on results from Biotype 100 and Fourier Transform Infra red spectroscopy (FTIR)). They were inoculated in the proportions found in the cheese. In parallel, a partition of 4 groups were empirically formed, each comprising 26 bacteria and 16 or 17 yeasts. Each group was used to make model cheeses. They were evaluated for proteolysis, lipolysis, aroma compounds and by sniffing. In the first analysis, cheese made with Groups 3 and 4, resulted in cheeses which gave very similar properties to the one obtained with the mixture of 83 strains.

In a series of 3 successive partitions, the mixtures were reduced to 2 groups containing 6/7 bacteria and 4/5 yeasts. These two associations gave cheeses which compare favorably with those made with the more complex associations, when organoleptic and biochemical analyses are considered. The use of molecular methods (PCR-SSCP) also permitted following the growth of the main species growing in the different mixtures.

This new way of screening seems to be very promising to study microbial interactions in cheese.

Keywords: cheese ecosystem, surface flora, screening method

P146 DOES THE DIVERSITY OF MESOPHILIC LACTOBACILLI IN RAW MILK DETERMINE THEIR GROWTH KINETICS IN COMTÉ CHEESE?

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The diversity of the sensorial characteristics of mature cheeses made from raw milk has often been related to the diversity of the raw milk microflora observed at the strain level. But the ways strain diversity influences these characteristics have not yet been investigated in detail. Mesophilic lactobacilli are one of the major non-starter cheese microflora originating from milk.

Twelve Comté-type cheeses were manufactured at a rate of three cheeses per week according to an experimental design. Manufacturing and ripening processes only differed by the addition (3 × 3 cheeses) or not (3 cheeses) of raw milk microflora isolated from milks of three different origins in the base milk (microfiltered) at the beginning of manufacture. Mesophilic lactobacilli were enumerated on FH medium. Their strain and species diversity was established after strain typing and species affiliation of isolates by PCR methods.

Mesophilic lactobacilli grew in all cheeses. They reached a higher density in the cheeses inoculated with raw milk microflora, similar in all these cheeses. But they exhibited different growth kinetics which were specific to each raw milk microflora and due to differences in their initial growth rates. Results strongly suggest that the growth kinetics of mesophilic lactobacilli in Comté cheese were largely determined by the initial cell density of *Lactobacillus paracasei* and *Lactobacillus rhamnosus* strains in milk, but also by the presence of different strains for both species in each raw milk microflora responding differently to the conditions prevailing at the beginning of manufacture.

Keywords: mesophilic lactobacilli, diversity, growth kinetics, Comté cheese

P147 RIPENING AND SEASONAL CHANGE IN MICROFLORA AND PHYSICO-CHEMICAL CHARACTERISTICS OF THE EWES' CHEESE PECORINO ABRUZZESE

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Pecorino Abruzzese is a typical and distinctive ewes' semihard cheese produced in Abruzzo (region of central Italy) closely associated with the territory. In this work, changes in microflora and in some physico-chemical characteristics during cheese-making and maturation process were studied. Pecorino cheeses were manufactured according to traditional procedures in the same dairy farm with raw or pasteurized milk, in summer and in spring. A total of four batches were studied, and samples were collected at 0, 14, 30 and 60 days of ripening. The number of coliforms in the cheeses was rather high at start of ripening and progressively reduced, but they did not disappeared at the 60th day of ripening. Coagulase positive staphylococci and pseudomonas had similar behaviour. Lactic acid bacteria evolution is reported in figure; in summer higher numbers than in spring were counted and different trends were observed. Enterococci ranged from 10^4 to 10^6 cfu/g at the beginning of ripening and generally increased in all the cheeses. In spring yeasts showed high numbers at the start of ripening (10^3–10^4 cfu/g) and then sharply decreased; in summer they had an opposite trend. Physico-chemical parameters such as pH (6.84–4.96), chloride content (14.2–29.6 g/kg) and a_w (0.998–0.880) were also determined. Cheese fatty acids were examined and quantitative differences were observed, particularly in oleic acid. The results suggest the necessity to increase the hygienic quality of Pecorino Abruzzese during the whole productive process, and to use selected autochthonous LAB starters.

Keywords: Pecorino Abruzzese, microbiota, ripening, fatty acids

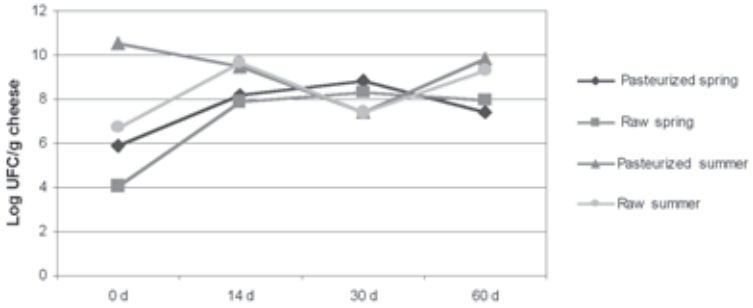

Figure 1: Evolution of lactic acid bacteria during ripening in the four batches

P148 NEW INSIGHTS INTO RED-SMEAR CHEESE RIPENING COMMUNITIES: FROM CULTURABLE APPROACHES TO DNA-BASED OVERVIEWS

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The use of defined starters is now a common practise to improve both the safety and the organoleptic qualities of ripened cheeses. Nevertheless, the composition of the smear community is still not restricted to the inoculated flora. It may include members of the

microbial community resident in the factory as well as occasional food contaminants, food pathogens or not. As recently demonstrated in the literature, diversity and species composition of the ripening microflora may also interfere with the occurence of food-born pathogens. It is therefore essential to be able to monitor it using appropriate methods.

Cultivation approaches allow us to identify the diversity of the culturable fraction of the microflora only, biaising our view of the microbial diversity. However, recently introduced rDNA-based technology, which includes among other strategies cloning-sequencing of 16S rDNA genes amplified from whole community DNA and its fingerprinting variants hide new unsuspected bias.

The spread plate technique and cloning-sequencing of the 16S rDNA gene were applied to the caracterization of the bacterial surface flora of two red-smear cheeses, one being produced traditionally in farmhouse, the other provided by a cheesemaking industry using defined inocula. Emphasis was given on the differences obtained between the different approaches: Cultivation methods lead preferentially to the detection of aerobic culturable organisms, while inactive but living cells as well as unculturable cells could not be isolated. Conversely, using molecular methods, we detected cultured, uncultured cells as well as inactive cells, but also dead cells, which mislead our understanding of the microbial populations present at the cheeses surface. We are therefore coming to the conclusion that bias of cultivation methods are replaced by bias of molecular methods.

Both methods used in the study are informative and complementary, and the need to combine them is clearly illustrated.

Keywords: Bacterial diversity; red-smear cheese; Cultivation; 16S rDNA cloning,

P149 INFLUENCE OF THE TYPE OF LACTIC STARTER CULTURE ON GROWTH AND ACTIVITY OF RIPENING CULTURES ON A CHEESE MODEL

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Aerobic cheese model previously developed by Chr Hansen was used to simulate a ripened smeared soft cheese, in order to evaluate the influence of the type of lactic starter culture on the growth and activity of various ripening cultures.

Cheese model was inoculated with lactic starter either only consisting in *Lactococcus lactis* subsp. *lactis biovar diacetylactis* („D" type), or only consisting in *Lactococcus lacti*s subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* („O" type). The surface of the cheese model was then inoculated on its surface, with a mix of ripening yeasts (*Debaryomyces hansenii*) and bacteria (*Brevibacterium linens* and *Staphylococcus xylosus*). Microbial population, pH and aspect of the cheese surface were evaluated up to 30 days of incubation at 12 °C.

The type of lactic starter culture used clearly influences the expression of some ripening cultures. While no difference in yeast growth was observed, the growth of the bacteria (*B. linens* and *S. xylosus*) was higher with the „D" type lactic starter than with the „O" type one. This resulted in a faster and more intense pigmentation of the surface of the cheese inoculated with *Lactococcus lactis* subsp. *lactis biovar diacetylactis*

This study demonstrates that selecting the right combination of lactic cultures and ripening cultures is a leverage to get the right speed and rate of ripening of the cheese.

Keywords: starter culture, ripening culture, pigmentation

P150 NATURAL FUNGAL FLORA INHIBITING *LISTERIA MONOCYTOGENES* ON CHEESE RIND

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Cheese, except the long ripening ones, could permit survival and growth of *Listeria monocytogenes*, which can grow at refrigeration temperatures in slightly acid substrates and tolerates salt, being able to grow in 10% NaCl.

In Italy this micro-organism is particularly searched on „Taleggio" and „Gorgonzola" cheeses. These cheeses are characterized by the presence of inoculated selected moulds belonging to genus *Penicillium* (in „Blue Cheese" and „Gorgonzola") and by spontaneous surface microflora development during ripening (yeasts, moulds and *Micrococcaceae* in smear and mould cheeses as Camembert, Brie, Taleggio).

The main objectives of the present research were to detect the natural fungal flora species which colonize mould cheeses and to verify if these species could inhibit *L. monocytogenes* strains isolated from cheese rinds.

Eight *L. monocytogenes* strains and 5 *Penicillium* species were isolated from 68 mould cheese rind samples of different origin and identified.

Fungal species were inoculated in broth and incubated at room temperature for 5 days. Mycelium was separated from supernatant by filtration and sterile filter paper disks were wetted with fungal filtrates and placed on ACS (a cultural medium which simulates a mould cheese rind) previously inoculated with *L. monocytogenes* strains. After incubation, plates were observed to detect inhibition halos.

Two strains of *L. monocytogenes* showed the most resistance to metabolites and only 1 strain was strongly inhibited by all fungal species. *P. griseofulvum* and *P. commune* metabolites had the most inhibiting activity on all *Listeria* strains. Metabolites of other fungal species had variable activity; in particular *P.crustosum* inhibited 4 *L. monocytogenes* strains, *P. aurantiogriseum* 3 (the most sensitive) and *P. chrysogenum* only 2 strains.

In conclusion, eumycetic flora could influence *L. monocytogenes* growth, but the sensitivity to moulds varies among strains.

An effective inhibiting action could be obtained by preservation of the natural environmental ecosystem present in traditional ripening caves.

Keywords: *Listeria monocytogenes*, Moulds, cheese rind, inhibition

P151 BEHAVIOUR OF *LISTERIA MONOCYTOGENES* IN RAW MILK CHEESES

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The objective of the study was to determine the development of *L. monocytogenes* in an artisanal raw milk cheese, the Registered Designation of Origin Saint Nectaire cheese produced in Massif Central area in France. Saint Nectaire cheeses manufactured with raw milks originating from six different farms were inoculated with two strains of *L. monocytogenes* (5 to 10 cfu/25mL of milk). Microbial and chemical analyses were carried out at appropriate intervals during ripening. The bacterial community was also analysed by SSCP-PCR (Single Strand Conformation Polymorphism-PCR).

In the cheeses core, the growth of *L. monocytogenes*, identical whatever the strain, was only observed during the first days of manufacturing in 3 cheese productions. No growth was observed in cheeses, manufactured with milks from 3 farms, which pH was lower than 5.3. However, a pH value of 5.3 can be considered as high compared with inhibitory values often mentioned in the literature. L-lactate concentrations in cheeses at 8 days were higher than 12 mg/g and could be also involved in the inhibition. In spite of differences in bacterial counts on different media, no correlation was observed with the inhibition of *L. monocytogenes.* Each cheese production had its own microbial community as visualised on SSCP profiles and at least one peak was associated with the early inhibition.

The growth of *L. monocytogenes* on the rind was different from that in the core as it occurred between 8 and 18 days and was only inhbited in two cheese productions.

Further studies will be performed to understand why the population of *L. monocytogenes* remains stable during ripening of raw milk cheeses. The role of the microbial community will be checked by comparing the growth of *L. monocytogenes* in raw milk and microfiltered milk cheeses.

Keywords: *Listeria monocytogenes*, inhibition, raw milk cheese

P152 TECHNOLOGICAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM „FORMAGÈLA VALSERIANA", A TRADITIONAL ITALIAN CHEESE

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Several factors influence the development of the organoleptic characteristics of the cheese: type and microbiological quality of milk, cheese-making technology, ripening conditions and others. However, lactic acid bacteria (LAB) play a principal role in releasing specific compounds responsible for flavor development; their most important property is acid production, but also the indirect contribution to the texture. Another important parameter is the redox potential (Eh), which contributes to the microbial quality, creates a balanced flavor development and protects compounds from oxidation.

„Formagèla Valseriana" is a traditional Italian farmhouse cheese, manufactured from raw-milk, without addition of starter cultures in the alpine valley Valle Seriana. Ripening process takes place in natural caves for more than 30 days.

The aim of this study was to value and to characterize the dominant microflora of Formagèla Valseriana cheese produced in mountain and plain farmhouses.

Microbial changes were examined during the manufacture, after 1 and 30 days of ripening. Strains of indigenous LAB were isolated, identified and characterized according to their acidifying, proteolytic and reducing activities.

Analyses show high level (10^8 cfu/g) of many LAB species and interesting differences between microflora occurring both in curd and cheese and in mountain and plain samples. Differences in technological properties inside the species were found.

Lactococcus lactis ssp. *lactis* predominated, strains from curd showed lower acidifying and higher reducing activity. Other main species were *St. thermophilus* and *Enterococcus* spp. (none of the strains were resistant to vancomycin).

Lc. garviae and *E. durans* were isolated only from curd, while all *Lactobacillus* strains come from cheese.

Results suggest that specificity and peculiar sensory characteristics of traditional cheeses are strictly related to the high level of biodiversity of the indigenous microflora. Over the centuries the technologies of these cheeses have selected bacterial ecosystems so complex that it is impossible to reproduce them exactly and dangerous to wipe out.

Keywords: traditional cheeses, lactic acid bacteria, technological properties

P153 EFFECT OF VARIOUS FACTORS ON PROPIONIC ACID BACTERIA GROWTH IN THE MODEL MEDIA

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The medium was based on a water extract of 15 days old Swiss type cheese. The medium was used for modelling of various factors effecting the growth of propionic acid bacteria (PAB).

The effect of *Lb. rhamnosus* L4 metabolism products on the growth of four various PAB strains used for Swiss type cheese production was measured in this medium. Growth retardation caused by *Lb. rhamnosus* L4 metabolism products was less marked for quickly growing strains of PAB. Extent of PAB growth inhibition was also dependent on the content of living *Lb. rhamnosus* L4 cells during the exponential growth phase of PAB.

It has been indicated that PAB growth in the model media is significantly influenced by quantity of inoculum. The growth was normal at cell concentration higher than 10^7 cfu/mL, at starting numbers of 5×10^6 cfu/mL growth ocured only after 15 days of cultivation at 30 °C. No growth was observed at concentration lower than 10^6 cfu/mL during 30 days of cultivation at 30 °C.

The growth of propionic acid bacteria was also significantly affected by starting concentration of diacetyl and glutamic acid in the medium.

Keywords: propionic acid bacteria, growth inhibition, diacetyl, glutamic acid

P154 IN VITRO SURVIVAL OF PROBIOTIC BACTERIA CONTAINED IN A WHEY CHEESE VECTOR SUBMITTED TO A GASTROINTESTINAL ENVIRONMENTAL CONDITIONS

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The interest associated with the manufacture of a probiotic whey cheese should be backed up by knowledge of the survival profile of the probiotic bacteria inoculated, during passage through the gastrointestinal tract, so as to guarantee that those bacteria are able to adhere and colonize the colon at sufficiently high viable numbers.

Therefore, the survival and stability of probiotic bacteria inoculated in whey cheese, exposed to simulated gastrointestinal tract conditions, were duly evaluated. Such probiotic bacterial strains as *Bifidobacterium animalis*, *B. animalis* BB12, *B. animalis* Bo, *Lactobacillus acidophilus*, *L. acidophilus* Ki, *L. casei* and *Lactococcus brevis,* previously inoculated in whey cheeses, were exposed to conditions similar to those prevailing in the gastrointestinal tract; cells in sterile saline water 8.5 g/L at 37 °C were exposed to acid (pH 2.5–3.0) and pepsin (1000 units/ mL), and 3 g/L bile salts was subsequently added by 60 and 120 min.

All assayed bacterial strains maintained their viable cell numbers when exposed to the aforementioned artificial gastric juice. *Bifidobacterium animalis* BB12, *B. animalis* Bo and *Lactococcus brevis* presented the highest viable numbers even when exposed to 3 g/L bile salts. *Lactobacillus acidophilus* survived in the presence of the artificial gastric juice and the same added with bile salts, respectively, for 60 min (with a death rate of 0.1788 Log (cfu/g)/min) and for 90 min (with a death rate of 0.1455 log cfu/g/min). After 60 min of exposure to the artificial gastric juice, *L. acidophilus* Ki decreased its viable cell numbers during the first 30 min in contact with bile salts, exhibiting a death rate of 0.019 log cfu/g/min; the cell numbers were kept afterwards for 90 min. However, when bile salts were added following 120 min of contact with the artificial gastric juice, those bacteria died more rapidly, according to a death rate of 0.1124 log cfu/g/min.

Keywords: acid, bile salts, probiotic, whey cheese matrices

P155 EXAMPLE OF MICROBIAL AND BIOCHEMICAL KINETICS OF BACTERIA RIPENED SOFT CHEESES INOCULATED WITH DEFINED SUB-ECOSYSTEMS OF LIVAROT

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Experimental cheeses inoculated with two different sub-ecosystems of a Livarot including three yeasts and four surface bacteria isolated from industrial Livarot and were ripened for 76 days under aseptic conditions. Two typical floras were compared. Cheese A contained *Geotrichum candidum* (2 strains*), Candida catenulata, Brevibacterium linens*, *Corynebacterium*, *Staphylococcus* and *Brachybacterium* species and cheese B *Geotrichum candidum*, *Candida catenulata*, *Debaryomyces hansenii*, *Arthrobacter* (2 strains), *Staphylococcus* and *Brevibacterium* species.

D. hansenii (cheese A) grew rapidly during the first two days, and then slowed but remained exponential until day 10 (generation time around 70 h). *G. candidum* (cheese A and B) growth mainly took place in the cheese surface with two or three exponential growth phases, depending on the strain used. *C. catenulata* (cheese A and B) seemed to have an important ripening role due to its important development. For *G. candidum* and *C. catenulata* the salting had an important effect on their growth, in relation to the strain used. *Staphylococcus* and *Brachybacterium* concentrations remained constant (around 10^6–10^8 cfu.g^{-1}) during the ripening but *Arthrobacter* sp. and *Brevibacterium* sp. (cheese B) but also *Brevibacterium linens* and *Corynebacterium sp.* (cheese A) have been shown to grow, all four, exponentially between d6 to d13 and remained constant between d15 and d76 (2.10^{10}cfu.g^{-1}). The spreading of these four strains largely prevailed over the growth of *Staphylococcus,* and *Brachybacterium*. Moreover, they were able to grow inside the cheese and reach 10^8 cfu.g^{-1}, in the center, at the end of ripening. Change in pH, in colour (measured by C* (chroma) and h° (hue angle) system) and creamy under rind thickness were followed. Cheeses A and B were different in pH and colour evolution. But both cheeses had the properties of a well developed Livarot cheese.

Keywords: yeasts, ripened surface bacteria, microbiology, biochemistry, Livarot type cheeses.

P156 A SURVEY OF THE *ENTEROBACTERIACEAE* ISOLATED FROM AN ARTISANAL ITALIAN EWE'S CHEESE (PECORINO ABRUZZESE)

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Fourty six *Enterobacteriaceae* were isolated from 4 batches of Pecorino Abruzzese, a traditional cheese produced in the Central Italy: they were a significant part of the microbial population of this cheese during the first part of ripening. In fact they ranged from 10^4 to 10^5 cfu/g in the curd, from 10^3 to 10^5 cfu/g after 15 days of ripening and after 60 days their number decreased but some of them were present (10^2 cfu/g). The dominant species at the start of ripening was *E. coli* (30 % of isolates), followed by *Salmonella arizonae* (26 %), *Serratia odorifera* (13 %), *Enterobacter sakazakii* (8.7 %), *Kluyvera* ssp. (4.3 %); *Escherichia coli* dominated also the end of cheese ripening. The isolates were identified and studied for some technologically relevant properties such as organic acid production, lipolytic and proteolytic activities and secondary compound production in milk. A great diversity on the phenotypic traits of the isolates were observed depending on the strains and the species, *Serratia* and *Escherichia* isolates showing a relevant proteolytic activities, and *Salmonella, Serratia* and *Enterobacter* lipolytic activity. Most part of the enterobacteria studied produced lactic acid (0.1–2.5 g/L) and acetic acid (0.45–3.4 g/L), whereas citric acid was completely consumed. A few were the aromatic secondary compounds with acetoin as the most significatively present.

Keywords: *Enterobacteriaceae*, Pecorino Abruzzese

P157 NSLAB DEVELOPMENT DURING RIPENING OF CACIOTTA CHEESES OBTAINED FROM RAW, HIGH PRESSURE HOMOGENIZED AND PASTEURIZED MILK

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The principal aim of this work was to compare cheeses obtained from cow milk previously subjected to a high pressure homogenization at1000 bar (HPH) with those produced from raw and heat treated cow milk. In particular, the effect of milk high pressure treatment on

the *Lactobacillus* population was investigated. The phenotypical and genotypical characteristics of 100 isolates, collected over a 27-days ripening period, were studied. According to the data obtained the HPH, in comparison with the other milk treatments, induced a modification of the quali- and quantitative composition of *Lactobacillus* population. All the cheese types, regardless of the treatment applied to milk, were dominated by *Lactobacillus plantarum* and *Lactobacillus pentosus* at the beginning of ripening. However, the HPH cheese were characterised also by the presence of heterofermentative species such as *Leuconostoc* ssp. Moreover, the HPH treatment of milk favoured the replacement during the ripening of *Lb. plantarum* and *Lb. pentosus* with species such as *Lb. casei* and *Lb. paracasei*. A key role in cheese aroma formation has been attributed to the latter species by a wide literature.

The causal agents of this microbial shift could be identified in the different ripening patterns shown in HPH cheeses with respect to the controls. In particular, the milk HPH treatment induced an acceleration of the proteolysis and lipolysis processes.

Keywords: Caciotta cheese, high pressure homogenization, *Lactobacillus*, ripening

P158 SAFETY TESTS FOR NEW *GEOTRICHUM CANDIDUM* STRAINS USED IN CHEESEMAKING

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Geotrichum candidum, an ascomycetous anamorph yeast-like fungus displaying phenotypic variability, is naturally a part of the raw milk microflora and is also used as a ripening agent of numerous cheeses. In some rare cases, pathological strains are known, causing geotrichosis. We were looking here for a correlation between potentially pathogenic and saprophytic strains of *G. candidum*.

Sixty one strains of *Geotrichum candidum* were chosen on the basis of the nature of the substrate from which they had been isolated, with emphasis on strains isolated from human substrate. They were genetically studied by RAM-PCR with the GATA4 probe (Gente *et al*., 2002). This molecular tool allows us to form four classes depending on the pattern profiles obtained. We could observed that the profiles correspond to four ecological niches (which we could also name „origin"): food (milk, dairy products…), human origin (stools, sputum, nail…), intermediate between food and human origin (stools), and environment (corn fodder, tomato, grass, malting environment…).

The behaviour of *G. candidum* towards gastrointestinal tract stress conditions was assessed. Physiological resistance and growth tests were realized in MEA medium modified, according to the test envisaged: Growth at 37 °C and 40 °C. Growth at pH 1.5 (buffer glycocolle-HCl). Growth with 1.5% Oxgall. The difference between resistance or growth under stress conditions was pointed out, if growing, the microorganism could be potentially invasive.

The development of a sensitive pathogenicity test is under study. It is based on *in vivo* pathogenicity testing on *Galleria mellonella* larvae and *in vitro* studies involving adhesion on Caco-2 cell line.

Keywords: *Geotrichum candidum*, physiological studies, safety tests.

P159 INTRACELLULAR AMINOPEPTIDASE AND ESTERASE/LIPASE ACTIVITIES OF *PROPIONIBACTERIUM ACIDIPROPIONICI* 4.6: INFLUENCE OF PH AND NACL

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The effect of pH and NaCl concentration on aminopeptidase and esterase/lipase activities of a *Propionibacterium acidipropionici* strain, isolated from a traditional Greek cheese, was investigated.

Enzymic activities in intracellular extract were detected spectrophotometrically and electrophoretically using synthetic substrates at pH 7.0 in absence of NaCl and in simulated traditional Greek cheese-like conditions eg. pH 5.5 and 7.5 % NaCl or pH 4.5 and 5.5 % NaCl.

Intracellular extract showed an aminopeptidase activity at pH 7.0 in absence of NaCl on all amino acid p-NA derivatives with the following order: Arg-pNA > Ala-pNA > Gly-pNA > Pro-pNA >> Lys-pNA > Leu-pNA > Met-pNA > Val-pNA. Aminopeptidase activity on all substrates at pH 5.5 and NaCl 7.5 % was reduced compared to the activity at pH 7.0. The activity decreased farther when assayed under pH 4.5 and 5.5 % NaCl except for Met-pNA, Pro-pNA and Val-pNA in which hydrolysis was higher than that at pH 5.5 with 7.5 % NaCl.

The electrophoretic pattern of aminopeptidase system was rather heterogeneous. The highest number of active bands was seen at pH 5.5 and 7.5 % NaCl. No activity was shown up on Pro-β-naphthylamide except the case of pH 4.5 and 5.5 % NaCl.

The esterase and lipase activities were detected on most of fatty acid derivatives but the higher activities were found against sort chain acids (C_2 to C_6). The majority of esterolytic activity was on α-naphthyl butyrate at pH 7.0. The presence of NaCl and reduction of pH increased the hydrolysis of some derivatives. In the case of α-naphthyl acetate and α-naphthyl caprylate the hydrolysis was higher at pH 4.5 and 5.5 % NaCl than in the other conditions.

The electrophoretic zymogram showed six individual active bands with R_f values from 0.18-0.30 using α-naphthyl derivatives as substrates. The strain was more active in simulated traditional Greek cheese-like conditions.

Keywords: propionibacteria, aminopeptidase, lipase.

P160 RESIDUAL ACTIVITIES OF PLASMIN/PLASMINOGEN AND RENNET ENZYMES IN CHEESE

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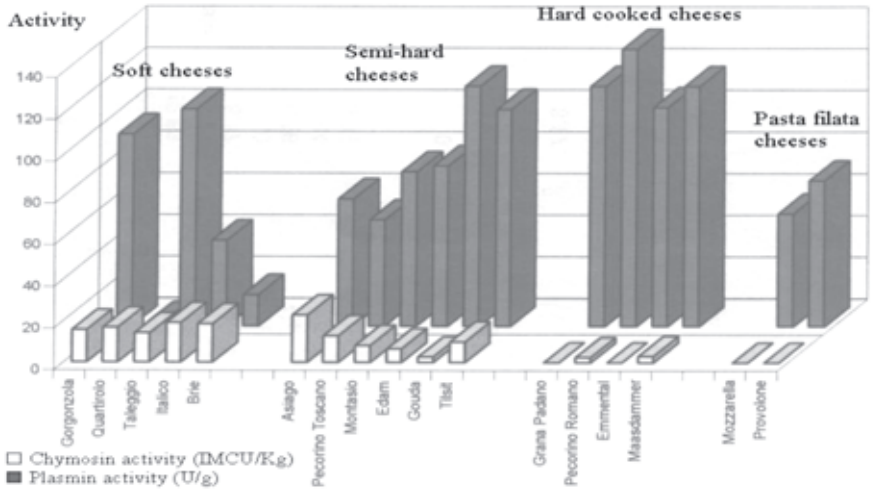
The activities of the two main proteinases involved in the primary degradation of casein have been monitored by specific analytical methods in experimental curds and commercial cheeses.

A spectrophotometric method based on evaluation of p-nitroaniline released by hydrolysis of a chromogenic substrate (D-Val-Leu-Lys-p-nitroanilide) was used to evaluate the activity of plasmin and plasminogen system. The residual activity of clotting enzymes was tested by RP-HPLC determination by using a synthetic heptapeptide (Pro-Thr-Glu-Phe-[NO_2 –Phe] -Arg-Leu) as substrate.

The technological parameters that mainly affect the activity of rennet and plasmin were found to be the pH of milk or cheese and the cooking conditions of curd. The lower the pH of milk was at renneting, the greater chymosin amount was retained in experimental curds (14% at pH 6.0 vs 6% at pH 6.6). Higher residual activities of rennet enzymes were observed in soft-type cheeses, while a negligible residual activity resulted in hard-cooked (e.g. Grana Padano, Pecorino, Emmental, Maasdammer) and in pasta-filata cheeses (Mozzarella, Provolone), probably due to thermal inactivation.

Conversely a severe cooking treatment causes a higher removal of plasminogen-inhibitors in whey, resulting in increased conversion of plasminogen to active plasmin (100-130 U/g). Soft, semi-hard and pasta-filata cheeses showed lower plasmin activities, especially in acid conditions (2 U/g in Quartirolo cheese), while higher pH values enhanced plasmin activity even in absence of cooking, as observed in Gorgonzola and Taleggio cheeses (92 and 104 U/g respectively).

Keywords: plasmin, rennet, activity, cheese.



P161 IMMUNOASSAYS FOR QUANTITATIVE MEASUREMENT OF PEDIOCIN PA-1 IN BIOLOGICAL SAMPLES

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Pediocin PA-1 belongs to the class II family of bacteriocins, a class of small, heat-stable, membrane-active peptides that is inhibitory for a broad spectrum of gram-positive bacteria, including spoilage and food-borne pathogens. The development of efficient immunochemical detection, quantification and purification methods for pediocin PA-1 would greatly facilitate the use of this bacteriocin as food preservative and enable its monitoring in foods and through the gastro-intestinal tract.

Polyclonal antibodies of predetermined specificity to pediocin PA-1 were generated after immunization of rabbits with two chemically synthesized peptides conjugated to carrier protein keyhole limpet hemocyanin (KLH). The chemically synthesized C-terminal peptide (22-44) and N-terminal peptide (1-9) represent the most and the less specific regions, respectively, with predicted immunogenic

properties. The same peptides conjugated to thiopropyl Sepharose have been used for affinity chromatography of highly pure non-nonsense antibodies. Three pools of early low avidity, medium high avidity and late highest avidity antibodies were obtained, all with high specificity.

The commonly used bioassay methods sometimes raise problems like lack of specificity and sensitivity. The highly sensitive sandwich ELISA method needs two pure antibodies specificities, one coated as capturing and one biotinylated, enzyme or fluorochrome conjugated as detector gave 500–1000 times more sensitivity. Attempt to make bacteriocin multiplex immunoassays are in progress.

Keywords: bacteriocin, Pediocin PA-1, Sandwich ELISA

P162 USE OF THE MILK ENZYMES FUCOSIDASE AND ALKALINE PHOSPHATASE AS INDICATORS OF THERMIZATION

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The introduction of low-heat treatments, such as thermization, in cheese making has created the need for further tests to ascertain the nature of heat treatment applied to milk. But at present, there is a lack of reliable methods for the control of products heated at sub-pasteurization temperatures.

International scientific literature is not profuse regarding this issue but a number of publications have identified the milk enzyme α-L-fucosidase (FC) as a possible marker of thermisation (57 to 68 °C).

Cheese being a very complicate living matrix, we decided to perform as a first step a feasability study on milk.

We optimized in our laboratory the reflectance colorimetric method published by Mc Kellar and Piyasena and also developped a spectrophotometric detection to measure the fucosidase activity. Both methods have been extensively tested to assess their analytical performances.

Inactivation curves of the fucosidase enzymatic activity have been established for cows and goats milk. Results obtained showed that, contrary to previous publications, fucosidase is inactivated at the very early stages of the thermization range.

Studies contacted in parallel within our laboratory lead to the conclusion that alkaline phosphatase (AP), another milk enzyme used to prove regulatory compliance of pasteurized milk, was subject to thermal inactivation for the upper level of the thermization process.

Consequently, we decided to attempt a combination of the two enzymes, fucosidase and alkaline phosphatase, in order to characterize milk samples having undergone thermization over the whole range of relevant temperatures.

We also present results of a study dealing with seasonal and regional variations of both enzymic markers in french raw milk.

This project has been partly conducted under our mandate as Community Reference Laboratory for milk and milk products.

Arilait (french association for the research in the dairy industry) was associated to our work through provision of the raw milk samples.

Keywords: thermised milk, raw milk, fucosidase, alkaline phosphatase

P163 RAPID IDENTIFICATION OF NSLAB ISOLATES FROM CHEDDAR CHEESE USING THE PCR LIGHTCYCLER SYSTEM

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The Non-Starter Lactic Acid Bacteria (NSLAB) flora of Cheddar cheese is dominated by mesophilic lactobacilli throughout most of the ripening period. The species most regularly encountered include *Lactobacillus paracasei, Lb. plantarum, Lb. curvatus* and *Lb. rhamnosus.* Significant evidence has accumulated to indicate that they play an important role in the development of cheese flavour. Identification of the lactobacilli to species level has generally been by biochemical methods, which are laborious and as they often rely on a limited number metabolic traits may be unreliable. More recently, molecular techniques that are more definitive and decrease the time required for identification have been developed. In this work, species specific primers in combination with real time PCR (LightCycler) have been used to further reduce the time taken to identify NSLAB isolates from cheese to species level. Using two well-characterised culture collection strains for each species, confirmation of the species-specific nature of the primers for *Lactobacillus paracasei, Lb.casei, Lb. plantarum, Lb. curvatus, Lb. rhamnosus* and *Lb. pentosus* was obtained. Forty-three isolates, selected from various Cheddar cheeses on LBS agar (which selects for lactobacilli), were tested using this technique. Some of these isolates had been previously identified using RAPD. Where isolates had previously been identified to species level, the results were confirmed in all cases with the LightCycler technique. Application of this technique enables purified isolates to be identified to species level within one working day.

Keywords: NSLAB, real time PCR, species level identification.

P164 PREPARATIVE IMMUNO AFFINITY CHROMAGRAPHY PURIFICATION OF BACTERIOCINS BASED ON SPECIFIC ANTIBODIES AGAINST SYNTHETIC PEPTIDES

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Pediocin PA-1 and Sakacin P are so-called class IIa bacteriocins produced by Lactic Acid Bacteria. Such bacteriocins are important natural bacterial inhibitors of interest for food preservation as they have a broad inhibition spectrum against food borne pathogens and spoilage organisms. Pure preparations are needed for the characterization of the bacteriocin and may also be useful for practical application. However, traditional purification methods are requiring a number of steps, and often activity is lost during purification. Here we describe a purification method for sakacin P and Pediocin PA-1 using immuno affinity chromatography.

Starting without pure immunogens (e.g. bacteriocins), bioinformatic simulated immunogenic peptides were first synthesized against the conservative N-terminal and polymorphic C-terminal part of the bacteriocins. The specific anti-peptide no-nonsense antibodies were purified by affinity chromatography using columns where the immunogenic peptides were coupled. The obtained antibodies proved to be highly pure by microfluidic protein electrophoresis (Agilent 2100 Bioanalyzer).

The reversed immuno affinity columns were made by coupling the specific antibodies to Sepharose 6B. Highly purified preparations of Pediocin PA-1 and Sakacin P were isolated from culture supernatants and their biological activity was tested in diffusion agar plate bioassays and by ELISA.

Keywords: bacteriocins, Pediocin PA-1, Sakacin P, purification

P165 IDENTIFICATION OF DAIRY RELATED *CLOSTRIDIUM* SPECIES BY AMPLIFIED 16S RIBOSOMAL DNA RESTRICTION ANALYSIS (ARDRA)

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In cheese manufacturing *Clostridium* spores can be a serious problem resulting in late blowing defects and bad taste. Identification of *Clostridium* species are usually conducted using a combination of physiological and morphological tests. These are time and labor consuming methods, while PCR based methods are faster and give a precise identification.

Computer analysis of published 16S rDNA sequences indicated that differentiation between the 4 most commonly isolated *Clostridium* species could be obtained by the ARDRA technique, using the restriction enzyme *Hha*I.

Practical tests using type and reference strains of *C. tyrobutyricum*, *C. butyricum*, *C. beijerinckii*, and *C. sporogenes* confirmed that a quick differentiation between these four species was possible. The identification procedure requires 4 handling steps (template preparation, PCR, enzyme digestion and gel electrophoresis) and can be conducted within one working day with a number of isolates.

Keywords: *Clostridium*, ARDRA, cheese

P166 PRODUCTION OF CHEESE RIPENING *LACTOBACILLUS CASEI* RECOMBINANT ENZYMES, AMINOPEPTIDASE AND ESTERASE

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The NSLAB was always predominated particularly by *Lb. casei* group (*Lb. casei/Lb. paracasei/Lb. rhamnosus*) in good quality of mature Cheddar cheese and they were judged to improve cheese flavor. *Lb. casei* group predominated in later stage of ripening, regardless of cheese types (in particular for semi-and hard type) and this flora is unlikely unique to each plant and each country. Cheddar cheese ripening was accelerated with the adjunct live *Lactobacillus casei* or shocked/lysis cultures, but some defects produced by the cultures look into the other avenue using the clean overepxressed enzymes. *Lactobacillus casei* ssp. *casei* LLG naturally expresses a high level of intracellular proteinase, aminopeptidase and esterase, and the combined activity of these enzymes resulted in the production of cheese flavour compounds. Further genetic studies were successful in overproducing the aminopeptidase and esterase in *E. coli* and *Pichia pastoris* up to 1,000 fold (about 50% of total cellular proteins), and a simple down stream process was developed to purify these enzymes, thus improving the economics and specific activity of the products.

Keywords: cheese ripening, *Lactobacillus casei,* recombinant enzymes, aminopeptidase, esterase

P167 EFFECT OF NEUTRASE® ON PROTEOLYSIS, BITTERNESS AND VISCOSITY OF ENZYME MODIFIED CHEDDAR CHEESE

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The objective of this study was to evaluate the effect of Neutrase® 0.5L (0.05, 0.1, 0.15, 0.20 and 0.25% v/w) on proteolysis, bitterness and viscosity of experimental enzyme modified cheddar cheese (EMC). Proteolysis was quantitatively determined by measuring the free amino groups in 5 % (w/v) phosphotungstic acid (PTA)-soluble fraction with an o-phthaldialdehyde assay method of Frister et al. (1989). The PTA-OPA value and bitterness increased with the concentration of enzyme preparation, while apparent viscosity of experimental EMCs decreased. Proteolysis and bitterness scores of experimental EMCs evaluated by members of a sensory panel showed a correlation coefficient of 0.987.

The correlation coefficients between proteolysis and apparent viscosity of EMC at temperatures of 40, 50 and 60 °C were 0.964, 0.943 and 0.902 respectively.

The viscosity model was constructed using three fixed factors namely, added enzyme concentration (as levels mentioned above), temperature (40, 50 and 60 °C) and speed of viscometer paddle (100, 200 and 300 rpm). The model revealed that there was no significant effect of speed variable on viscosity ($p < 0.01$). The final model involved expression of viscosity as a function of enzyme dosage and temperature ($R^2 = 0.994$).

Keywords: Neutrase®, Enzyme Modified Cheddar Cheese, EMC, Proteolysis, Bitterness, Viscosity

P168 INVESTIGATION OF FLAVOUR DEVELOPMENT IN ENZYME-MODIFIED CHEDDAR CHEESE

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A two-stage process was developed to assess the potential of selected proteolytic, peptidolytic and lipolytic enzymes for their ability to generate Cheddar-type enzyme-modified cheese (EMC). The substrate consisted of fresh Cheddar curd, water, butterfat and emulsifying salts. In stage one, four proteolytic EMC products were produced using four commercial proteinases derived from different microbial sources in combination with a commercial microbial peptidase. Proteinase dose rates were selected to generate comparable levels of primary proteolysis to a target commercial EMC, which had previously been identified as having a flavour profile closest to a mature natural Cheddar cheese. A fixed level of peptidase was used in each product. Distinct differences in proteolytic patterns of each EMC were evident. Sensory properties were found to differ significantly ($P < 0.05$) by ranked preference analysis. The EMC with the highest score was chosen as the substrate for stage two.

Four commercial lipases with different selectivity derived from either microbial or animal sources were added to the EMC selected from stage 1. Lipase dose rates were selected to produce comparable levels of lipolysis to the target commercial EMC. This could not be achieved in the EMC produced using the animal derived lipase which may have been due to the presence of proteolytic activity in this lipase preparation and/or product inhibition caused by high levels of free fatty acids at the interface between the fat and lipase. No significant difference (<0.05) was found between each EMC produced in stage 2 and the target commercial EMC by ranked preference sensory analysis, indicating comparable sensory attributes. However, descriptive sensory analysis identified some significant flavour and aroma differences, which were attributed to differences in the extent of proteolysis and lipolysis and differences in their free amino acid and free fatty acid profiles.

Keywords: Enzyme-modified cheese, proteinase, peptidase, lipase

P169 ENCAPSULATION TECHNOLOGY: OPPORTUNITIES IN CHEESE PRODUCTION

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A multitude of encapsulation techniques which have been developed in the past 20 years are available to coat substances of interest for the cheese industry, ranging from molecular size (e.g. flavour compounds, peptides, enzymes) to microscopic size (e.g. bacteria, starter

cultures, fibres, acids and salts). By designing appropriate coatings (lipophilic, hydrophilic or combinations), the release of such ingredients into a surrounding medium can be controlled by temperature, hydration, shear, pH, ionic strength. In cheese-making (washed curd type of cheese), this opens ways to

i. include otherwise water-soluble substances into the curd and prevent leakage into whey in a cost-effective and efficient manner
ii. include strongly flavoured compounds into cheese, e.g. for taste-masking in the case of unpleasant tastes or for controlling release of flavouring
iii. prevent interaction of compounds with starter culture for specified times

From an array of encapsulation techniques available at NIZO food research on pilot and industrial scale (spray-drying, fluid bed coating, spray chilling, coacervation), spray chilling and complex coacervation were selected to demonstrate potential benefits of encapsulates in the production of semihard cheese (Gouda type).

In this poster, we describe the application of encapsulated enzymes and volatile flavour compounds in Gouda type cheese. Encapsulates were prepared by fat coating techniques and coacervation, respectively. The release of the compounds and the effects on the cheese's properties were examined. An outlook on benefits and new product concepts when making use of encapsulation technology is given.

Keywords: encapsulation, Gouda type cheese, enzymes, ingredients

P170 EFFECT OF ADDITION OF ENZYME-MODIFIED CHEESE ON PROTEOLYSIS AND SENSORY PROFILES IN CHEDDAR CHEESE

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A method to accelerate the ripening and sensory development in Cheddar cheeses by the addition of dried enzyme-modified cheese (EMC) during salting was investigated. Three cheeses were made in triplicate, a control cheese (without EMC addition), and experimental cheeses supplemented with 0.25 or 1.0 g/100g EMC powder. Populations of starter and non-starter lactic acid bacteria (NSLAB), indices of proteolysis and flavour development by quantitative descriptive sensory analysis were monitored over a 6 month ripening period. Populations of NSLAB were slightly elevated in the experimental cheeses relative to the control cheeses. During ripening, concentrations of proteolytic indices and total free amino acids increased in all cheeses, with the highest levels detected in the experimental cheeses at all times. RP-HPLC chromatograms of the pH 4.6-SN extracts of experimental cheeses showed similar peptide profiles to the control cheeses at 1, 4 and 6 months of ripening, but not at 2 months. The different profiles observed at 2 months are possibly due to additional substrates available at this time due to the added EMC, which was hydrolysed by 4 months, thus normal rates of hydrolyses were again observed. Principal component analysis of the significantly different ($P < 0.05$) sensory attributes indicated that all the cheeses had similar flavour profiles. Hierarchical cluster analysis grouped the experimental cheeses at 4 months with the control cheese at 6 months, suggesting that the experimental cheeses had developed a flavour profile at 4 months similar to the 6 month old control cheese, reflecting significant acceleration in ripening due to the addition of EMC powder.

Keywords: EMC, proteolysis, acceleration, flavour improvement

P171 EFFECT OF ADDITION OF ENZYME-MODIFIED CHEESE ON FREE AMINO ACIDS, FREE FATTY ACIDS, VOLATILE COMPOUNDS AND SENSORY PROFILES OF CHEDDAR CHEESE

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The addition of enzyme-modified cheese (EMC) powder to Cheddar cheese at salting has been shown to accelerate flavour development. The objective of this work was to study the impact of such additions on the evolution of individual free amino acids (FAA), free fatty acids (FFA), volatile compounds and how they relate to the sensory profiles over the ripening period. Three cheeses were made in triplicate, a control cheese (without EMC addition), and experimental cheeses supplemented with 0.25 or 1.0 g $100g^{-1}$ EMC powder. The cheeses were evaluated at 1, 2, 4 and 6 months of ripening. Levels of FAA were slightly elevated in the experimental cheeses at 1 and 2 months of ripening with the main FAA being leucine, valine, phenylalanine, and lysine. However, by 4 and 6 months levels of FAA were higher in the experimental cheeses with histidine, leucine and glutamic acid reflecting the greatest increases. Levels of FFA increased up to 2 months but decreased by 4 and 6 months. C14:0, C16:0 and C18:1 were higher in the experimental cheeses at 2 months than in the control cheese. Principal component analysis of the volatile compounds indicated that while the profile changed during

ripening, similar profiles were observed between cheeses at the same stage of ripening. 2-pentanone, pentanal, 3-methyl-butanal, 1-pentanol and 2-butanone were associated with 6 month old cheeses and grouped with a 'mouldy', 'rancid', 'soapy', 'astringent' flavour, a 'pungent', 'sweaty', 'rancid', 'soapy' odour and the FFA C10:0. The younger cheeses were associated with 2-methyl-butanal, 2-hexanone, 2-butanol and carbon disulphide and grouped with a 'buttery', 'processed', 'lard' flavour, a 'buttery' odour and the FFA's C12:0, C14:0, C16:0, C18:0, and C18:1. The results suggest that the levels of FFA maybe contributing the most to flavour differences of these cheeses and can be attributed to the addition of the EMC powder.

Keywords: EMC, FAA, FFA, volatile analysis, acceleration, flavour improvement

P172 MANUFACTURING OF WHITE PICKLED CHEESE FROM THE FULL CONCENTRATED WHOLE MILK'S RETENTATE

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Influence of ultrafiltration of whole and skim milk on rennetability in the course of white pickled cheese manufacturing was investigated. Various concentrating factors have been tested as 3, 4, 5 and 5.5-fold. The renneting properties of the unconcentrated milk and the retentates (skim and whole milk's ultrfiltration retentates) were explained. The white cheese produced from unconcentrated whole milk via traditional industrial method was compared with the white cheese produced by whole milk's retentate (5.5-fold). The retentate's cheeses were salted with different methods, i.e. dry salting, brine salting and salt addition before renneting. The effect of the salting method on the texture of UF-cheese during the shelf life was determined. It was suggested that the production of the white pickled cheese from whole milk retentate (full concentrated) was more suitable than from skim milk's retentate and with conventional method which uses unconcentrated milk, actually.

It was found that the most convenient way for manufacturing of white pickled cheese was using whole milk's full concentrated (UF) retentate (5.5×) instead of using of skim milk's retentate and/or of lower concentration factors. The ultrafiltration of whole milk is more suitable than skim milk's retentate used for manufacturing of full fat or half-fat white pickled cheese because of the problems arised from the recombination of heavy cream to the skimmilk's retentate. It possibly causes the change of fat/protein ratio and the total solids content of the cheese is never reached to the legal limits. Brining is the most effective method for salting of the cheese due to the textureal changes during the first 3 months of ripening. For industrial purposes, two salting methods are suitable because of the packaging practice of the white cheese; salting of retentate before rennet addition or brining of cheese, but salt in milk (or retentate) has got a risk factor which was related with the main biochemical changes it causes. Finally it is shown that the manufacturing of white pickled cheese by using of whole milk's full-concentrated retentate is possible, but the process has to be investigated in detail for explaining the overall modification which contribute to creating an industrial process.

Keywords: white pickled cheese, ultrafiltration, surface hydrophobicity, texture

P173 UTILIZATION OF PROTEIN CONCENTRATES IN THE MANUFACTURE OF MOZZARELLA CHEESE

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Whey protein concentrates (Dairy-lo) and sodium caseinate were used for the manufacture of Mozzarella cheese. Addition of 1 % of these concentrates gave cheese with acceptable organoleptic properties. The chemical and physical properties, microstructure and organoleptic properties of Mozzarella were assessed in fresh cheese and after 4 weeks storage at 10°C. Mozzarella cheese supplemented with caseinate gave the highest yield and gained highest score for flavour, body and texture compared to control and cheese supplemented with Dairy-lo. Control cheese showed the best meltability and oiling off. In all cheeses, meltability, firmness and oiling off, 12 % TCA soluble nitrogen, free tyrosine and tryptophan and volatile free acids increased significantly during cold storage. Electron microscopy (EM) was used to study the microstructure. EM showed in cheese with added sodium caseinate a heavy accumulation for casein matrix compared to the other cheeses. Oiling off, meltability and firmness changed significantly ($P < 0.05$) during the storage period.

Keywords: protein concentrates, Mozzarella cheese

P174 INCORPORATION OF POLYSACCHARIDES OF MICROBIAL AND NON-MICROBIAL ORIGIN IN CHEESE SYSTEM

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When the fat content of the Mozzarella cheeses is reduced, the moisture retention also decreases giving a low yield and poor melt and stretch characteristics. The cheeses develop rubbery texture, and increased hardness. Excess of calcium has been linked with excessive curd firmness. Polysaccharides of microbial and non-microbial origins have been used to increase moisture content and texture and melt characteristics of Mozzarella cheeses. Many strains of lactic acid bacteria, including *Streptococcus thermophilus* synthesise extracellular exopolysaccharides (EPS). EPS may remain attached to the cell wall (capsular EPS) or may be excreted into the surrounding medium (ropy EPS). EPS helps retain moisture in Mozzarella cheeses. This presentation will highlight use of microbial exo-polysaccharides produced by *S. thermophilus* 1275 and maltodextrin based Maltrin® M040 and M100 and modified potato starch based StaSlim® 143 polysaccharides on increasing moisture content and improving yield, texture and functional characteristics of low fat Mozzarella cheeses.

Skim milk Mozzarella cheeses were made using *S. thermophilus* 1275, two maltodextrin based (Maltrin® M040 and M100) and a modified potato starch based (StaSlimâ 143) polysaccharides which act as fat replacers. A control batch was made without any polysaccharides. The texture characteristics of the cheeses were measured using an Instron Universal Testing Machine. The location of the fat replacers in the cheese structure was examined using a SEM. There was an increase in moisture content with cheeses made with *S. thermophilus*1275. Maltrin® based cheeses did not show any increase in moisture content, while StaSlim® based cheeses showed reduced moisture levels. The hardness values of Mozzarella cheeses with Maltrin® were lower, while StaSlim® based cheeses were harder compared to control cheeses. During storage Maltrin® and control cheeses showed decreased hardness, cohesiveness, springiness, gumminess and chewiness values. The cheeses made with StaSlim® 143 showed increased hardness, gumminess and chewiness values during storage. StaSlim® formed smooth particles (about 0.05mm diameter) within the cheese matrix. Incorporation of Maltrin® and StaSlim® resulted in an increased openness in cheeses, and large serum channels (up to 0.1mm diameter) were seen. In general, cheese made with microbial EPS showed better yield and textural and functional characteristics.

Keywords: incorporation, polysaccharide, non-microbial origin, cheese

P175 MANUFACTURE OF PROVOLONE CHEESE BY USING DIFFERENT TYPES OF MILK

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Provolone cheese was made from standardized buffaloes, cows and goats milk (4.5 % fat) and from mixtures of buffaloes+cows, buffaloes+goats and cows+goats milk at a ratio of 1:1. The cheeses were stored for three months at 15 °C. The yield, chemical composition, physical properties and characteristics of the ripened products were evaluated. The cheese yield was the highest for buffaloes milk and the lowest for goats milk. Soluble nitrogen, soluble tyrosine and soluble tryptophan of cheese from buffaloes milk were lower than those of pure cows and goats milk. Cheese from buffaloes milk with 50 % of cows or goats milk showed higher values for soluble nitrogen, soluble tyrosine and soluble tryptophan and improved the firmness, flavour, body and texture of Provolone chesse.

Keywords: Provolone, cow, goat, buffalo milk

P176 EFFECT OF USING FAT REPLACERS ON CHEMICAL AND PHYSICAL PROPERTIES OF SOFT WHITE CHEESE

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Low fat soft white cheese was manufactured by using fat replacers to increase the moisture content and to improve the functional properties of the cheese. Protein based fat replacer (Dairy-lo) and carbohydrate based fat replacer (Inulin) which was extracted in laboratory from Dahila sp. were used at the rate of 50 % of the replaced fat. The cheeses were stored at 5 °C for 4 weeks. All cheeses were analyzed fresh and after 4 weeks for acidity, pH, fat, moisture, ash, soluble nitrogen, total volatile acids (TVA), tyrosine (tyr), tryptophan (trp), total carbohydrates, cholesterol, free amino acids (FAA) and organoleptic properties. Using fat replacers caused in fresh cheese a decrease in total energy, cholesterol, fat content and pH while moisture, acidity, total carbohydrates, ash, total protein and TVA content

of cheese were increased. During cold storage of soft cheese for 4 weeks moisture, total nitrogen and pH were decreased whereas acidity, fat, ,TVA, tyr, trp, total carbohydrates and cholesterol were increased. Cheese with fat replacers obtained good scores and did not affect cheese quality.

Keywords: low-fat cheese, fat replacer

P177 UTILIZATION OF MICROFILTRATION ON THE PRODUCTION OF EWE'S MILK CHEESE

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This study was carried out to evaluate the effects of different treatments of raw ewe's milk on the physical, chemical, microbiological and sensorial properties of cheese. The bulk raw milk was divided into three equivalent portions and the first was pasteurised (72 °C; 50 s) (PM), the second was defatted, microfiltered (1.4 µm Membralox GP™ multichannel ceramic membrane) and finally mixed with pasteurised cream (65 °C; 30 min) (MFM+PC) and the third was not processed (RM). The experiment was performed in triplicate and physical, chemical and microbiological analyses were carried out in cheeses at 1, 10, 20 and 30 days of ripening. The sensory properties of the cheeses were evaluated after a ripening period of 30 days.

Microfiltration effectively removed more than 99.9 % of total aerobic mesophiles, 99.9 % of spores and 98.3 % of somatic cells of skim milk. The composition in protein and total solids of MFM+PC was significantly lower ($p < 0.05$) from that of raw milk. No significant differences were observed on the chemical composition of the three types of cheese, except for fat on dry matter and moisture on defatted cheese between RM and MFM+PC. Cheeses manufactured with MFM+PC were also significantly different from those produced with raw milk in terms of total aerobic mesophiles, lactic acid bacteria and *Enterobactericeae* counts. Sensory analysis showed significant differences between raw milk cheeses and both PM and MFM+PC cheeses. The highest sensorial score was given to cheeses made with pasteurised and microfiltered milk, due to the more compact structure and stronger flavour of RM cheeses. Cheeses produced with microfiltered milk showed lower yield than RM and PM cheeses.

From this work it can be concluded that cross-flow microfiltration of ewe's milk ensures hygienic quality of „traditional" cheeses and holds potential for developing ewe's cheeses with good textural and sensory properties.

Keywords: microfiltration, ewe's milk, cheese

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P178 A MECHANISM DESCRIBING THE PROTEOLYTIC STAGE OF RENNETING OVER A BROAD RANGE OF CASEIN MICELLE CONCENTRATIONS

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At the natural acidity of milk the kinetics of the enzymatic stage of renneting may be adequately described as a first order reaction with the rate constant proportional to the enzyme concentration [*E*]

$$\frac{dS(t)}{dt} = -k \cdot S(t) = -k_0[E] \cdot S(t),$$

where $S(t)$ is the number of casein macro peptide hairs remaining on the surface of the casein micelles at time t after rennet is added to milk. At fixed calcium ion and enzyme concentrations the rate constant k decreases with increasing casein micelle concentration in milk. For instance, the value of k decreases when making cheese from ultrafiltered milk. While the dependence of the proteolytic reaction rate on the enzyme concentration is easily understood considering the enzyme's role as a catalyst, the reason for the influence of casein micelle concentration remains less clear.

We propose the following scheme for the proteolytic stage of renneting:

$$E(+M) \underset{1/\tau_C}{\overset{1/\tau_D}{\rightleftarrows}} (E_C) \xrightarrow{r} P$$

where t_D is the mean time an enzyme molecule spends between two successive collisions with micelles and t_C is the mean time the enzyme molecule spends in contact with a micelle. We assume that only the enzyme molecule may reversibly change its state after collision with

a micelle *M*, thus becoming an 'enzyme in contact' E_c, and that the micelle remains (essentially) the same micelle available for the association with a second (third, *etc*.) enzyme molecule. Moreover, the enzyme molecule may not become free after producing a product, but continue cleavage at rate *r*.

Based on this scheme we have shown that the rate constant is closely proportional to $\varphi^{-\frac{1}{3}}$ over a broad range of protein concentrations relevant to both traditional and UF-cheesemaking, thus explaining the experimentally determined decrease of the value of the rate constant with increasing casein micelle concentration. We have also shown that at very low micelle concentrations the power law dependence vanishes, and at very high concentrations it tends, at least hypothetical, to φ^{-1}.

Keywords: renneting, proteolysis, kinetics, UF-cheesemaking.

P179 EXOPOLYSACCHARIDE-PRODUCING *STREPTOCOCCUS THERMOPHILUS* – APPLICATIONS IN COTTAGE AND CHEDDAR CHEESE MANUFACTURE

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The functional effects of exopolysaccharide (EPS) producing lactic acid bacteria are well documented in yoghurt type products.

Here we report data from new commercial applications of an EPS strain in cottage and cheddar type cheese manufacture.

In Cottage cheese curd production, it increases yield, reduces fines and improves curd texture. In the dressing, initially applied to non-fat products to increase viscosity and improve mouth-feel, it is now used for all dressing types, providing superior cling effect on the curd, enhanced mouth-feel and reduced syneresis during shelf life.

In reduced fat Cheddar cheese, we obtain improved mouth feel (perception of creaminess) and increased water content, which softens the body and improves yield.

In standard Cheddar, it improves yield, commercially important in mild and economy Cheddar, where water content is maximised.

In mild Cheddar, curd particle melding is accelerated, so cheese is ready for cutting 12 days after manufacture.

In mature Cheddar, functionality is optimised, where sliceability has been compromised due to the characteristic texture shortening associated with maturation.

Keywords: exopolysaccharides, *Streptococcus thermophilus*, Cottage, Cheddar

P180 COMPARISON OF CHEESE MAKING PROPERTIES OF MILK FROM INDIVIDUAL COWS OF SWEDISH AND DANISH DAIRY BREEDS

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Milk proteins are of great importance for the quality of cheese and other fermented dairy products. The caseins are of particular significance because of their contribution to the structure and consistency of cheese. Casein amount and composition are closely related to milk-clotting properties and cheese yield.

The purpose of this work was to compare the cheese making properties of milk (e.g. milk-clotting properties and cheese yield) from individual cows of different dairy breeds and relate those to factors such as total concentrations of protein, milk fat, urea and casein. In further studies the protein composition will be examined more in detail and related to these results.

Milk samples (10 L) were collected from 16 Swedish red and white cows, 15 Swedish Friesian cows and 14 Danish Holstein-Friesian cows. Untreated milk samples were analysed for concentrations of total protein, fat, urea and casein. Cheeses were made from 4 liters of defatted and heat-treated milk (72 °C for 15 sec). Immediately after rennet addition (1.25 mL/L of Chy-max Plus, Christian Hansen, Denmark) rheological measurements were performed on a BohlinVOR Rheometer (Metric Analys AB, Stockholm, Sweden). The milk-clotting time and the elastic modulus G´ was related to the concentration of total protein, milk fat, urea and casein. Cheese yield was measured after two weeks of ripening in cool storage (10 °C) and related to the same parameters.

Preliminary results show that the higher concentrations of protein in milk from Swedish red and white cows and Danish Holstein-Friesian cows are related to higher cheese yield. There are also some divergent data, showing high concentrations of milk protein but low cheese yield and *vice versa*. Later we hope to find a correlation between those deviations and variations in casein composition. Any obvious correlation between the casein concentration *versus* clotting time and the elastic modulus has surprisingly not yet been found.

Keywords: milk proteins, casein concentration, milk-clotting properties, cheese yield

P181 EFFECT OF PASTEURIZATION TEMPERATURE ON THE COMPOSITION, RHEOLOGY AND FUNCTIONALITY OF HALF-FAT CHEDDAR CHEESE

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Recent evidence suggests that *Mycobacterium paratuberculosis,* which is responsible for Johne's disease in cattle, could survive conventional pasteurization of milk at 72 °C for 15 s, especially if the count in the raw milk is > 10^4 cfu/mL. Consequently, it is now widespread commercial practice to pasteurize at > 72 °C. However, little is known about the effect of higher pasteurization on the texture and cooking properties of cheese. Cheddar cheese containing half the fat of normal Cheddar (Half-fat Cheddar; approximately 15 % fat)) was manufactured from milk pasteurized at 72, 77, 82 or 87 °C for 26 s. The mean levels of whey protein denaturation in the milk were 2.8, 8.4, 20.2 and 34.1 % of total whey protein, respectively. The cheeses were ripened at 4ṣC for 30 days and subsequently at 8 °C for 330 days. Increased pasteurization temperature significantly increased the levels of moisture and decreased the levels of protein, fat and calcium in cheese. Increasing the milk pasteurization temperature had little or no effect on primary proteolysis (as measured by pH 4.6-soluble nitrogen) but significantly increased the levels of secondary proteolysis (as measured by 5 % phosphotungistic acid-soluble nitrogen). Increased pasteurization temperature significantly reduced fracture stress, fracture strain and firmness of cheese. The flowability and stretchability of the melted cheese were not significantly effected by increasing pasteurization from 72 to 77 °C but were markedly reduced on further increasing it from 72 to 82 or 87 °C.

Keywords: pasteurization temperature, whey protein denaturation, half-fat Cheddar, Composition, Rheology, Functionality

P182 EFFECT OF PASTEURIZATION TEMPERATURE AND TIME ON CHEDDAR CHEESEMAKING EFFICIENCY

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Pasteurization of cheesemilk at > 72 °C for 15 s has become widespread practice in recent years. Increasing pasteurization temperatures (PT) gives higher cheese moisture and therefore has the potential to increase cheese yield. Full-fat Cheddar cheese was manufactured from milk pasteurized at 72, 74.6, 75.9 or 78.5 °C in a plate heat exchanger; for each PT the milk was held for 15, 25, 35 and 44 s. Cheesemaking was standardized with respect to starter-to-casein ratio, rennet-to-casein ratio, firmness at cut (54 Pa), cooking/stirring regimes, and pH at different stages of manufacture. Increasing PT increased whey protein denaturation, from ~ 5 at 72 °C to ~ 14 at 78 °C for a holding time of 15 s, and increased the time required to attain the standard cut firmness. The moisture content of the cheese increased significantly with PT, at a rate of ~ 0.4 % (w/w) per 1 °C for a holding time of 15 s. Fat loss to cheese whey, as a % of total milk fat, increased with PT from ~ 11 at 72 °C to ~ 17 at 78.5 °C. The actual cheese yield, expressed as kg per 100 kg of milk normalized to a protein plus fat content of 6.65 %(w/w), increased significantly from 9.6 kg at 72 °C to ~ 9.8 at 78.5 °C. However, the moisture-adjusted cheese yield decreased from ~ 9.9 to 9.6 kg over the temperature range, owing to the simultaneous decrease in fat recovery to cheese. Compared to PT, increasing the holding time had only relatively minor effects.

Keywords: pasteurization temperature, whey protein denaturation, Cheddar cheese, composition, yield

P183 EFFECT OF GEL FIRMNESS AT CUTTING ON CHEDDAR CHEESEMAKING EFFICIENCY

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Cutting the rennet-induced gel is a central part of cheese manufacture, being the first step in the dehydration process by which the fat, protein and other colloidal constituents are concentrated. In modern cheese plants, gel firmness at cutting can vary substantially, especially where there are large seasonal variations in milk composition. This requires that the set-to-cut time be regularly updated so that the target cut-firmness is achieved. Cheddar cheeses were made in 500 L cheese vats and the firmness of the gel at cutting was varied from 0.5 to 80 Pa. Otherwise, cheesemaking was standardized with respect to starter-to-casein ratio, rennet-to-casein ratio, cooking/stirring regimes, and pH at different stages of manufacture. The study demonstrated that rennet-induced milk gels could be cut at a range of firmness values while giving satisfactory cheese manufacture. Gels cut at very low firmness (e.g. 0.5–2 Pa) were very soft and „liquid-like", and appeared as if the transition from a liquid to a gel was not yet complete. Yet, these „under-set„ gels gave satisfactory cheese

composition and fat recoveries. This suggests that curd particles from gels, which are cut when very soft, undergo rapid firming during the cutting and early stirring stages. Increasing the firmness at cutting from 0.5 to 80 Pa increased cheese moisture content (~ 3 %, w/w), cheese yield (~ 0.4 kg/100kg milk), and the level of fat lost to whey at firmness values > 40 Pa. In contrast, increasing the cut firmness decreased the levels of fat and protein in the cheese, and cheese pH.

Keywords: gel firmness, cutting, Cheddar cheese, composition, yield

P184 INFLUENCE OF MILK PROTEIN FORTIFICATION ON CHEDDAR CHEESEMAKING EFFICIENCY

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Standardization of milk protein content and protein-to-fat ratio can minimize the impacts of seasonal variations in milk composition on cheesemaking efficiency. The current study evaluated the effects of standardizing the milk protein from 3.3 % (w/w) (control, CTL) to 4.0 % (w/w) by ultrafiltation (UF) or by the addition of phosphocasein (PC; 84 % protein) or milk protein concentrate (MPC; 87 % protein), on Cheddar cheese. Milk was ultrafiltered to ~ 14 % protein at 50 °C; PC or MPC were dispersed in freshly skimmed milk to give a final added protein level of 10 % (w/w). The protein-fortified skim milks were then blended with whole milk and cream to give standardized milks with 4 % protein and a protein-to-fat ratio of 0.96. Standardized milks (500 L) were pasteurized at 72 °C × 26 s and cheesemaking was standardized with respect to starter-to-casein ratio, rennet-to-casein ratio, firmness at cut (54 Pa), cooking/stirring regimes, and pH at different stages of manufacture. Increasing milk protein level significantly reduced moisture level but gave increases in fat recovery to cheese (from ~ 89 to 91.5 % total milk fat), actual cheese yield (Ya, from ~ 9.5 to 11.6 kg/100kg milk) and moisture-adjusted cheese yield (to 38.5 %, w/w) (Yma; from ~ 9.7 to 12.1 kg/100kg milk). The yield increase with PC-fortified milk was higher than that expected from the higher levels of fat and protein in the PC-fortified milk, as reflected by the higher actual yield per 100 kg milk adjusted to a fat plus protein level of 6.7 % (w/w), Yafpam. The Yafpam, in kg/100kg normalized milk, increased from 9.53 for CTL to 9.72 for the PC milk. Cost benefit analysis indicated a net financial benefit on using PC to standardize cheesemilk to 4 % protein.

Keywords: milk protein fortification, cheese yield, Cheddar

P185 PREDICTING THE CUTTING TIME OF COTTAGE CHEESE USING A BACKSCATTER SENSOR

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Cottage cheese manufacture involves the mesophilic fermentation of skim milk to pH Ł 4.8, cutting the gel before cooking, washing and addition of cream dressing. Cottage cheese is sometimes made with the addition of a low concentration of rennet and as a result the gel is ready for cutting at a higher pH value. Since the cutting time is a critical factor influencing the quality of cottage cheese an objective method to determine when the gel should be cut is desirable. The effects of gelation temperature (23, 27 and 32 °C) and inoculation rate (0.5, 2.75, and 5 %) on the gelation properties of reconstituted 10 % (w/w) skim milk made with the addition of 2 mg/kg rennet was studied. A completely randomized block design with three replicates was performed. The gelation of milk was monitored using an inline backscatter sensor operated at 880 nm and the cutting time (t_{cut}) of milk gel was evaluated visually using a spatula. The pH of the gels at cutting time was recorded. The effects of temperature and inoculation rate on the cutting time were evaluated by surface response plot. Multiple regressions between the cutting time and backscatter parameters were used to generate prediction algorithms. Time based parameters collected from the backscatter sensor were useful for prediction of gel cutting time, e.g., t_{2min2} which was the time to the second minimum of the second derivative of the light backscatter ratio profile. One simple algorithm, $t_{cut} = b_1 t_{2min2}$ predicted the cutting time with a SEP = 6.61 min and $R^2 = 0.999$. Gelation temperature and inoculation rate significantly ($P < 0.001$) affect the t_{cut} as does their interaction term ($P < 0.05$). The results of this study indicate that a backscatter sensor could be a useful, nondestructive and objective method to determine when to cut cottage cheese gels.

Keywords: cottage cheese, cutting time, backscatter, gelation

P186 INVESTIGATION OF JOINT COAGULATION OF MILK AND CHEESE WHEY PROTEINS FOR SOFT CHEESE TECHNOLOGY

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Modern industrial soft cheese technology has both, advantages (low milk consumption, simple technology) and disadvantages (insufficient utilization of dairy components).

Theoretical principles of cheese technology improvement are based on the great biological potential of non-separated whey from soft and brine cheese. First of all, this potential is characterized by biologically valuable proteins from whey and surface of fat globules. The next reason is the presence of casein and milk fat. As we see it, using of cheese whey will not only improve the biological value, but also increase protein and fat isolation degrees in the production of soft cheese and solve the problem of rational processing of dairy whey as well.

The most interesting in this aspect is creation of low-waist cheese technology using joint thermo-acid coagulation of milk and cheese whey proteins. During our research we examined and found out the following:

1. Mutual influence of physical/ chemical content of raw materials and activity of joint protein coagulation. Dry matter content, protein content and level of acidity of raw materials play the main role. High efficiency of coagulation and maximum yield of cheese mass are watched by the highest dry matter content and acidity of raw mixture 18–22 °T.
2. Influence of active acidity, temperature, duration time on protein and fat sedimentation degree. Optimum parameters: active acidity 5.0–5.2, temperature 94–98 °C, duration time 30 minutes.
3. Dynamics of whey protein, fat and casein transmission from raw mixture into cheese mass. It is established that in optimal zone we can catch 100% of fat, 86% of whey proteins and 66% of casein particles.
4. Composition of raw mixture and its influence on yield and sensor parameters of cheese. Optimum initial ratio milk: cheese whey – 40:60.
5. New cheese composition, alimentary and biological value. Digestion rate – 98.9%, protein utilization – 89.8%, biological value – 98.6%, vital amino acids content – more than 100%.

The results have become a solid ground for new industrial technology of soft cheese with increased whey protein content.

Keywords: soft cheese, thermo-acid coagulation, whole milk, cheese whey

P187 MINERALISATION EVOLUTION AND BUFFERING CAPACITY IN A SOFT CHEESE CURD DURING CHEESEMAKING

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The milk composition and parameters of cheesemaking (rennet coagulation, acidification and drainage) are important for controlling the biochemical characteristics, structure and rheological properties of the curd at mould removal. Ripening and consequently the final quality of cheese are very depending on these parameters.

Two manufactures of soft cheese curds were carried out; the first was done from milk containing two casein concentrations and the second at two renneting pH values. During these manufactures, biochemical characteristics (contents in nitrogen, fat, dry matter, mineral in the total and in the aqueous phases, buffering capacity) were determined.

The analysis of results obtained indicates that concentration in casein and values of renneting pH have significant influences on the mineralisation evolution during manufacture. Consequently, the biochemical characteristics such as the content in minerals and the buffering capacity of the curd at mould removal were affected. Globally, the curds have high mineralisations and buffering capacities when the casein concentration in milk and the renneting pH values were high.

Keywords: soft cheese, acidification, draining, minerals

P188 INFLUENCE OF GENETIC POLYMORPHISM ON THE TECHNOLOGICAL PROPERTIES OF CHEESE-MILK

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During recent years there have been several studies reporting effects of genetic protein polymorphism on the technological properties of cheese-milk. Milk protein variants are responsible for both milk clotting properties as well as the cheese yield. In this study, the influence of genetic polymorphism of β-casein, κ-casein and β-lactoglobulin, on protein distribution between curd and whey during cheese making is going to be analysed. Furthermore, the impact of the different genetic variants of milk proteins on the rheological properties of chymosin induced milk gels will be studied.

Cows from the university experimental herd were genotyped for β-casein and κ-casein using the PCR-based Pyrosequencing® method, while Fast Protein Liquid Chromatography (Äkta® FPLC) was used for β-lactoglobulin genotyping. Milk samples from individual genotyped animals were clotted using a solution of chromatographically pure chymosin and subjected to syneresis to form a „micro fresh cheese". The whey fractions and the curd will be analysed, both after clotting and after syneresis, for concentrations of α_{s1}-, α_{s2}-, β-, κ-casein, β-lactoglobulin and α-lactalbumin, using Äkta®FPLC and Capillary Zone Electrophoresis. Any correlation between the concentrations of individual proteins and milk protein genotype will be studied. HPLC will be used to detect and monitor proteins and protein degradation products. So far the rheological properties of milk gels have been analysed using a Bohlin VOR Rheometer. The course of coagulation was monitored after adding a solution of chromatographically pure chymosin to the milk samples.

The preliminary results indicate that milk containing κ-casein AE/β-casein A1A2 has negative and the κ-casein BB/κ-casein A1B positive impact on the coagulation start time and elastic module G'. However, these results are yet to be confirmed on a larger number of animals and analysed for the impact of the genetic variants of β-lactoglobulin.

Keywords: genetic polymorphism, milk proteins, milk clotting properties, cheese yield

P189 EXOPOLYSACCHARIDE PRODUCTION BY *LACTOBACILLUS ACIDOPHILUS* FOR POTENTIAL APPLICATIONS IN FRESH CHEESE

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Many strains belonging to the group of lactic acid bacteria (LAB) produce exopolysaccharides (EPS). The ability of EPS to act as viscosifying, stabilizing, and/or water-binding agents in various foods makes it an effective natural alternative to commercial synthetic stabilizers, so it may play an important role in the dairy industry. In fact, EPS-producing LAB are not only important in manufacture of yoghurt and fermented milks, but also in cheese production. Recent research efforts have shown that EPS cultures are useful to increase moisture retention and improve functional properties of low-fat Mozzarella cheese and whey cheese. In this work, the ability of *Lb. acidophilus* to produce EPS, and the performance of two different methods for EPS isolation was investigated.

Fermentations were carried out in a Braun Biostat B 2-L fermentor, filled with 1.5 L of MRS broth containing 20 g/L lactose. The yield of the EPS isolation method was tested by adding xanthan gum to the medium. The experiments were carried out at 37 °C, 150 rpm, pH 5.5 under an N_2 atmosphere. A 50 mL/L standard inoculum was prepared from a subculture of *Lb. acidophilus*, previously grown in the corresponding medium for 20 h at 37 °C, and was used to start-up every fermentation batch. Fermentation batches took 48 h, and samples were taken periodically. Growth was monitored spectrophotometrically and by plate enumeration. To optimise EPS isolation, cells and residual polypeptides were removed by centrifugation (4000 rpm for 20 min) upon precipitation via two different processes: addition of pronase E solution and one volume of 20 % trichloroacetic acid, or precipitation with 20 g/kg 5-sulfosalysilic acid. The EPS was precipitated by three volumes of cold ethanol and was collected by centrifugation. The weight of isolated and dried polymers was measured, and the total amount of carbohydrates was determined by the phenol-sulphuric method. Exponential growth took place for ca. 12 h, and EPS was produced mainly during the stationary phase.

Keywords: exopolysaccharides, *Lb. acidophilus*, lactic acid bacteria

P190 LACTO-DYNAMOGRAPHIC STUDY OF LACTIC ACID BACTERIA AS A TOOL FOR THEIR SELECTION

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The lacto-dynamograph provides, through a diapason tracing, a graphic image of the milk coagulation mechanisms by means of three parameters, that are correlated to several curd characteristics: rennet coagulation time (r in min), curd firming rate (k_{20} in min) and curd firmness (a_{30} in mm) measured 30 min after the addition of rennet. This allows a simple and quick identification of animals that produce over-reactive or under-reactive milk, regarding rennet.

Lactic acid bacteria are involved in the production of a variety of fermented foods. The selection of starter cultures for cheesemaking is performed on the basis of numerous characteristics, also in some way referred to their role in cheese rheology.

The aim of this research has been to develop a method to directly screen lactic acid bacteria regarding their coagulation properties using the lacto-dynamographic technique. Therefore, several autochthonous strains of lactic acid bacteria, previously isolated from Italian raw milk cheeses, have been inoculated in pasteurised goats' and ewes' milk and studied using a lacto-dynamograph to determine their behaviour.

The lacto-dynamographic analysis of lactic acid bacteria-inoculated milks shows different types of tracing and, on the whole, an interesting biodiversity among the tested strains.

This simple and quick screening method could give the opportunity to easily perform a pre-selection of a high number of strains, discriminating lactic acid bacteria regarding their coagulation properties.

Keywords: lacto-dynamographic analysis, coagulation parameters, lactic acid bacteria, pre-selection

P191 INFLUENCE OF GOAT MILK ADDITION ON FETA CHEESE'S YIELD

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According to the Commission Regulation no. 1107/96 Feta cheese is produced with ewe's and goat's milk. The maximum amount of goat's milk which can be used in feta cheese production is thirty percent. The current research investigated the effect of varying goat's milk content on the feta yield and composition.

Two factory-scale feta cheese productions were derived from five different goat's and ewe's milk ratios. The applied milk ratios were 100 % ewe's milk, 10 %, 20 %, 25 % and 30 % goat's milk. The yield from each cheese had been calculated on 56 % cheese moisture and for 8 different cheese ages.

Feta cheese produced from pure ewe's milk has the highest yield at 120 days (which aggrees with Mallatou et al., 1994) and the cheese with 30 % goat's milk the lowest yield at 120 days. At the age of 30 days, all the mixtures, apart from the pure ewe's milk, have their optimum yield. Conversely, feta cheese produced with small quantities of goat's milk had higher yields. Goat's milk content also affected he firmness of the cheeses produced (agreeing with previous work, Tsigkros *et al*, 2003).

Keyword: Feta cheese, goat milk addition, yield

P192 NEW INNOVATIVE PROCESS FOR CHEESE MAKING

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A new, extremely simple process for cheese making with concentrated milk has been developed at ALP: raw, standardised milk is microfiltered through a membrane with pores of 100 nm. Key factors are the temperature, pressure and flow conditions, whilst the pressure difference across the whole membrane is kept constant (UTP). During microfiltration, the casein and fat are concentrated. A concentration factor of 5–8 (depending on the desired dry matter in the cheese) results in a concentrate, which can be used directly for producing soft or semi-hard cheeses.

After the addition of cultures (starter and surface culture), rennet extract (ca. 0.2 ‰) and sodium chloride (ca. 1 %), the concentrate is poured into the desired mould and coagulated for 5–20 minutes at 35–38 °C. After coagulation, the mould is turned out, the cheese is

acidified and then ripened. Any desired ingredients can be added before coagulation and the cheese can be divided into portions after coagulation.

Microfiltration (100 nm pores) instead of Ultrafiltration (5–80 nm pores) allows much more favourable concentration (greater flux, smaller filter area) and a higher final concentration, so that semi-hard cheeses without curd formation can also be produced. Native whey proteins are only little concentrated. Using diafiltration, if necessary combined with specific thermal pretreatment of the raw milk, the percentage of whey proteins and also the lactose concentration in the vat milk can be precisely regulated. The percentage of whey proteins in the cheese is therefore dependent on heating of the milk, as in traditional cheese making. With the new process it becomes possible to make cheese which is very similar to traditionally made cheeses in terms of composition and quality. The loss of fat and casein in the permeate is negligible.

In future, full concentration by means of microfiltration could become the key technology for continuous soft and semi-hard cheese production. The advantages are obvious:

- less investment in plant and equipment
- labour-saving production
- smaller quantities of rennet and cultures per kg of cheese
- significantly better flux performance than with UF-concentration
- continuous production without curd formation
- continuous mould filling and portioning
- high-grade permeate: no copper, no rennet, no glucomacropeptide (GMP), unacidified, practically free of microorganisms, with native whey proteins
- higher yield: no loss of casein and fat through permeate and whey
- innovative cheese possible in any mould with a variety of ingredients
- cheese comparable with conventionally made products in terms of quality and composition

Keywords: cheese, liquid-filled, microfiltration, concentration, fractionation

P193 LOW-FAT CHEESE WITH INCORPORATION OF WHEY PROTEINS

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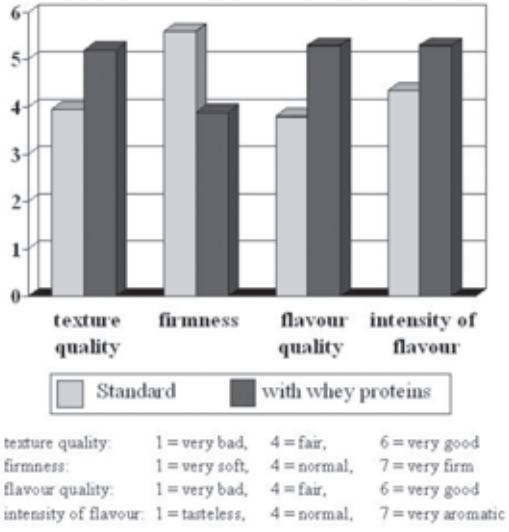
Low-fat cheese has very often a firmer and longer texture as well as a less intensive flavour. In a new newly developed process, whey proteins are precipitated with heat and lactic acid, homogenized and added to the milk before cheese making.

The cheese with added whey protein particles has a softer texture and a more intensive flavour. The incorporation of whey proteins led to a distinctly higher water content and as a consequence, higher yield. The costs of raw material were decreased by 7 – 10 %. The higher intensity of flavour would allow a 20–40% reduction in ripening time. The higher sensory qualities open up the possibility of a higher market price.



Keywords: cheese, low-fat, whey protein, flavour, texture, yield

P194 CLARIFYING INVESTMENT IN A CENTRAL RIPENING STOREHOUSE FOR HARD CHEESE

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Decisions concerning the investment needed to replace installations in a cheese-ripening storehouse (Thurgau Cheese-Ripening Co. Ltd.) were examined in a diploma thesis. Three different versions were compared from the points of view of cost calculation and efficiency analysis.

The aim of this report was to examine the logistics from the making of the cheese to its delivery to the loading platforms, and to critically compare new/other alternatives. Suggestions for the amount of investment needed to replace the cheese-treating facilities were also to be defined, and a look taken at technical feasibility and financial repercussions. A comparison of the various alternatives was undertaken by means of a cost comparison calculation and an efficiency analysis.

Three possible versions were compared. The first involves investment in cheese-treating facilities and new plastic pallets, as well as a lorry with special cheese-loading devices. During storage, the cheese can be regularly treated by means of the centralised treating facilities.

In the second version, the cheese is collected using conventional lorries with lifting platforms and covered racks. For storage, the cheese must be stacked on special storage shelves. Here the cheese can also be cleaned regularly by means of central treatment facilities.

For the third version, the logistics are handed over to an external transport company. The cheese is stored on rotating shelves and ideally not cleaned during fermentation. Only following fermentation, when the cheese has been cooled in the storage cellar, is it cleaned and turned over by means of mobile robots.

Both the cost comparison calculation and the efficiency analysis speak for maintaining the pallet system. On the basis of these findings, it seems wise to apply the first version, practicable within a two-year time frame.

Keywords: logistics, cheese-treating facilities, efficiency analysis

P195 A METHODOLOGY TO ASSESS THE PRESENCE OF ANGIOTENSIN-CONVERTING ENZYME-INHIBITORY DIPEPTIDES: APPLICATION TO ABONDANCE CHEESES

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The angiotensin-converting enzyme-inhibitory (ACEI) activity in cheeses is mainly due to oligopeptides: 73 of the 159 identified ACEI fragments present along the sequence of α_{s1}-, α_{s2}-, β- and κ-casein are dipeptides. Since these dipeptides are repeated 1 to 8 times along the sequence of caseins, they correspond to only 27 different dipeptides.

This allowed to develop a methodology based on the comparison of the elution times of these synthetic ACEI dipeptides with the elution patterns of fractions rich in dipeptides of cheeses, using reversed-phase high performance liquid chromatography (RP-HPLC). The fractions rich in dipeptides were prepared by size exclusion HPLC of cheese water soluble extracts.

This methodology was applied to an Abondance cheese sample. The fraction rich in dipeptides was analysed by RP-HPLC: the retention times of the peaks in the chromatogram obtained were compared with the retention times of the 27 ACEI dipeptides considered. When peaks had retention times close to those of ACEI dipeptides, known quantities of these ACEI dipeptides were added to the fraction rich in dipeptides prior to RP-HPLC analysis. Following RP-HPLC analysis, 2 ACEI dipeptides were co-eluted with peptides present in the fraction, suggesting that they are present in the analysed cheese. Since these dipeptides were present in cheese at concentrations close to the concentration inhibiting 50% of the activity of ACE *in vitro* (IC_{50}), these dipeptides would thus have the potential to exert a physiological antihypertensive effect.

Keywords: cheese, angiotensin-converting enzyme inhibition, peptides, high performance liquid chromatography (HPLC)

P196 HIGHER CHEESE YIELD AND LOWER PROCESS COST

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With ever increasing cheese production there is a large demand for increased quality and production efficiency. In answer to this, NIZO food research has developed tools that help the industry to increase yield by more than 10 % and decrease the processing costs. This

improvement is reached by using the tool NIZO Premia. NIZO Premia is a computer platform technology that allows industry to calculate product properties and optimise process conditions. The complete cheese manufacturing is described by a modular and flexible process model. With coupled product models (e.g. protein denaturation, enzyme inactivation, combined salt and moisture diffusion, coagulation) all production steps can be monitored. In the Netherlands most of the cheese is direct or indirectly produced with the aid of NIZO Premia. Examples of processing steps for which a significant improvement could be achieved are:

- Product losses change-over milk/water (50% reduction)
- Membrane filtration (optimum process)
- Pasteurisation (protein aggregation and a decrease of fouling)
- Coagulation process (reduced coagulation time, optimal and constant gel strength)
- Extraction process (stirring curd-whey mixture: shorter time and up to 20% reduction in water)
- Draining and filling of curd (capacity and optimal process)
- Fermentation (optimal pH values in the cheese during processing)
- Salting/ripening of cheese (optimal storage conditions, taste control)
- Whey treatment (20% less fouling in equipment)

In conclusion, NIZO Premia has a proven track record, tested and validated in a wide range of cheese companies. Results: increased cheese yield, improved production efficiency reached with the same process configuration and the same of improved cheese quality.

Keywords: improved cheese yield, process model, product model, pasteurisation, coagulation, extraction, draining, ripening

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